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THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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VOLUME LXXIII

AUGUST TO DECEMBER, 1937

Smithsonian Institution
311924
National Museum

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INSTRUCTIONS TO AUTHORS

Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

Preparation of Figures. The dimensions of the printed page (4⅜ x 7 inches) should be borne in mind in preparing figures for publication. Drawings and photographs, as well as any lettering upon them, should be large enough to remain clear and legible upon reduction to page size. Illustrations should be planned for sufficient reduction to permit legends to be set below them. In so far as possible, explanatory matter should be included in the legends, not lettered on the figures. Statements of magnification should take into account the amount of reduction necessary. Figures will be reproduced as line cuts or halftones. Figures intended for reproduction as line cuts should be drawn in India ink on white paper or blue-lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc. must be in India ink. Figures intended for reproduction as halftone plates should be grouped with as little waste space as possible. Drawings and lettering for halftone plates should be made directly on heavy Bristol board, not pasted on, as the outlines of pasted letters or drawings appear in the reproduction unless removed by an expensive process. Methods of reproduction not regularly employed by the Biological Bulletin will be used only at the author's expense. The originals of illustrations will not be returned except by special request.

Directions for Mailing. Manuscripts and illustrations should be packed flat between stiff cardboards. Large charts and graphs may be rolled and sent in a mailing tube.

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THE

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THE MARINE BIOLOGICAL LABORATORY

THIRTY-NINTH REPORT, FOR THE YEAR 1936—FORTY-NINTH YEAR

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II. ACT OF INCORPORATION

No. 3170

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

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VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1936.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1936, the book value of the Endowment Funds in the hands of the Central Hanover Bank and Trust Company as Trustee, was

General Fund,	Securities	$ 910,572.21
	Real Estate	9,037.32
	Cash	1,616.08
Library Fund,	Securities	172,261.84
	Cash	21,584.91
		$1,115,072.36

The income collected from these Funds was as follows:

General Endowment	$41,941.24
Library Fund	8,251.32
	$50,192.56

an increase of more than $2,000 over the income from these Funds in 1935.

The income due from these Funds in arrears, some of which may never be collected, was on December 31, 1936

General Fund	$12,605.25
Library Fund	5,050.00
	$17,655.25

The total amount in arrears was about $325 less than on December 31, 1935.

The dividends from the General Biological Supply House have continued—the total received for the year amounting to $12,700.

Retirement Fund. A total of $4,060 in pensions was paid. The Fund at the end of the year consisted of securities of the book value

of	$18,923.27	
Cash	684.04	$19,607.31

Income in arrears on December 31st was .... $ 312.75

Plant Assets. The land (exclusive of Gansett and Devil's Lane tracts), buildings, equipment and library, represented an investment

of	$1,755,892.28	
less reserve for depreciation	471,880.72	$1,284,011.56

Income and Expenses. Expenses including $41,782.21 depreciation exceeded income by $6,951.86.

There was expended from current funds net $26,319.79 for plant account.

During the year the Laboratory acquired by gift from Dr. Meigs Lot "X," Bay Shore property and the bathhouse on it and by purchase the Howes property, completing the frontage on Water Street, and the assets of the Bar Neck Corporation which included the leasehold and buildings of the Penzance Garage and the adjacent Spindell lot. At the end of the year the Laboratory owed $8,500 on mortgage on the Howes property and $8,500 in notes given for the acquisition of the Bar Neck assets. In addition it owed on notes and accounts payable $10,855.71. It had accounts and notes receivable of $14,367.37 and $14,773.65 in cash and bank accounts in its current assets.

Following is the balance sheet, the condensed statement of income and outgo and the surplus account all as set out by the accountants:

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1936

Assets

Endowment Assets and Equities:		
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee—Schedules I-a and I-b	$1,115,072.36	
Securities and Cash—Minor Funds—Schedule II	8,298.06	$1,123,370.42

Plant Assets:			
Land—Schedule IV	$ 109,749.39		
Buildings—Schedule IV	1,238,562.84		
Equipment—Schedule IV	157,202.67		
Library—Schedule IV	250,377.38	$1,755,892.28	
Less Reserve for Depreciation		471,880.72	
		$1,284,011.56	
Cash in Dormitory Building Fund		223.24	
Cash in Reserve Fund		24.65	$1,284,259.45
Current Assets:			
Cash		$ 14,773.65	
Accounts and Notes-Receivable		14,367.37	
Inventories:			
Supply Department	$ 41,039.96		
Biological Bulletin	12,179.92	53,219.88	
Investments:			
Devil's Lane Property	$ 43,633.13		
Gansett Property	5,614.49		
Stock in General Biological Supply House, Inc.	12,700.00		
Securities and Cash—Retirement Fund—Schedule V	19,607.31	81,554.93	
Prepaid Insurance		3,293.50	
Items in Suspense (Net)		323.44	$ 167,532.77
			$2,575,162.64

Liabilities

Endowment Funds:			
Endowment Funds—Schedule III	$1,114,980.01		
Reserve for Amortization of Bond Premiums	92.35	$1,115,072.36	
Minor Funds—Schedule III		8,298.06	$1,123,370.42
Plant Liabilities and Funds:			
Mortgage—Payable, Howes Property		$ 8,500.00	
Notes—Payable a/c Bar Neck Property Purchase		8,500.00	
Donations and Gifts—Schedule III		1,032,072.61	
Other Investments in Plant from Gifts and Current Funds		235,186.84	$1,284,259.45
Current Liabilities and Surplus:			
Accounts—Payable		$ 5,317.04	
Notes—Payable		5,500.00	
Woods Hole Oceanographic Institution		38.67	
		$ 10,855.71	
Current Surplus—Exhibit C		156,677.06	$ 167,532.77
			$2,575,162.64

EXHIBIT B

MARINE BIOLOGICAL LABORATORY INCOME AND EXPENSE, YEAR ENDED DECEMBER 31, 1936

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund ...		$ 41,941.24		$ 41,941.24
Library Fund		8,251.32		8,251.32
Instruction	$ 8,176.73	10,305.00		2,128.27
Research	4,146.96	14,215.00		10,068.04
Evening Lectures	88.95		$ 88.95	
Biological Bulletin and Membership Dues	9,133.99	9,087.00	46.99	
Supply Department—Schedule VI	38,262.35	43,144.91		4,882.56
Mess—Schedule VII	21,588.75	20,443.21	1,145.54	
Dormitories—Schedule VIII ..	31,984.12	11,965.84	20,018.28	
(Interest and Depreciation charged to above three Departments—See Schedules VI, VII, and VIII)	35,320.19			35,320.19
Dividends, General Biological Supply House, Inc.		12,700.00		12,700.00
Rents:				
Danchakoff Cottages	294.76	700.00		405.24
Newman Cottage	100.96	250.00		149.04
Janitor's House	21.76	240.00		218.24
Howes Property	323.37	40.00	283.37	
Bar Neck Property		2,508.74		2,508.74
Sale of Duplicate Library Sets		250.79		250.79
Interest on Notes-Receivable ..		165.00		165.00
Sundries		50.90		50.90
Maintenance of Plant:				
Building and Grounds	23,393.18		23,393.18	
Chemical and Special Apparatus	13,329.83		13,329.83	
Library Department Expense	7,753.23		7,753.23	
Truck Expense	910.27		910.27	
Sundry Expense	173.58		173.58	
Workmen's Compensation Insurance	509.71		509.71	
General Expenses:				
Administration Expenses	14,912.47		14,912.47	
Endowment Fund Trustee ...	980.29		980.29	
Bad Debts	663.53		663.53	
Reserve for Depreciation	41,782.21		41,782.21	
	$183,210.81	$176,258.95	$125,991.43	$119,039.57
Excess of Expenses over Income carried to Current Surplus—Exhibit C		6,951.86		6,951.86
		$183,210.81		$125,991.43

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1936

Balance, January 1, 1936			$152,246.38
Add:			
Reserve for Depreciation charged to Plant Funds			41,782.21
			$194,028.59
Deduct:			
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,			
Land		$10,146.34	
Buildings		13,329.91	
Equipment		5,421.95	
Library		14,757.42	
		$43,655.62	
Less,			
Notes and Mortgage payable on account of additions to Plant, Land and Buildings	$17,000.00		
Received for Plant Assets disposed of	287.50		
Adjustment of Accrued Charges on account of Library, December 31, 1935	48.33	17,335.83	
		$26,319.79	
Pensions and Allowances Paid	$ 4,060.00		
Expenses on account of Retirement Fund Securities	905.43		
	$4,965.43		
Less,			
Retirement Fund Income	$ 845.13		
Profit on Sale of Retirement Fund Securities	40.42		
	$ 885.55	$ 4,079.88	
Excess of Expenses over Income for Year as shown on Exhibit B		6,951.86	37,351.53
Balance, December 31, 1936—Exhibit A			$156,677.06

Respectfully submitted,

LAWRASON RIGGS, JR.,
Treasurer

V. THE REPORT OF THE LIBRARIAN

A report of the budget assigned to the Library by the Executive Committee for the year 1936 is as follows: books, $1,000 (with the understanding that any part of this not used for books be transferred to back sets); serials, $6,000; binding, $1,500; express, $300; supplies, $500; salaries, $7,150; back sets, $2,350; total, $18,800. The sum of $250.79 acquired by the Librarian by the sale of duplicates increased the total budget to $19,050.79. The expenditures of the Library under the same headings are itemized for the end of the year as follows: books, $695.02; serials, $5,471.00; binding, $1,614.85; express, $187.10; supplies, $386.65; salaries, $7,150; back sets, $3,478.86; total, $18,983.48. An unspent balance of $67.31 may be accounted due to the sale of duplicates and the Librarian hopes that this sum in reverting to the general accounts may be used by the Laboratory toward securing a drying unit for the basement room in the dormitory where the Library's duplicate reprints are stored.

The acquisitions of the Library during 1936 follow: 34 back sets were completed and 20 partially completed; 29 of the completed sets were for the Marine Biological Laboratory and 5 for the Woods Hole Oceanographic Institution; 12 of the partially completed sets were for the former and 8 for the latter; the total number of current serial titles received was 1,339: 376 purchased by the Marine Biological Laboratory (19 new), 36 by the Woods Hole Oceanographic Institution (1 new), 630 by exchanges with the "Biological Bulletin" (9 new) and 57 by exchange for the publications of the Woods Hole Oceanographic Institution (18 new), 240 by gift, 224 and 16 respectively. The Marine Biological Laboratory purchased 86 new books and the Woods Hole Oceanographic Institution purchased 1, authors and publishers (authors, 8 and publishers, 26) presented 33 to the former and 1 to the latter; 30 were old books variously acquired and a fine copy of Swammerdam's "Book of Nature," 1758, was sent to the Marine Biological Laboratory by J. C. Waller of Liverpool, England, accompanied by a letter of good will to the Laboratory. The new reprints filed were 3,339 (675 current and 2,664 of previous date). Uncatalogued, and therefore omitted from this count, 580 further 1936 reprints are on hand, making the excellent total of 1,255 current reprints received before February of the following year. This is the highest record for any year in the history of the Library, and justifies the innovation of last summer in the reading-room display of current reprints. About 1,000 of the older reprints filed were from the large collection of Dr. Gilman A. Drew's reprints. Mrs. Drew presented this collection to the Marine Biological Laboratory

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last summer. There remain from the valuable collection more than 6,000 reprints duplicate to the files in the Library stacks. These will be placed in the duplicate collection.

With the final statement of this report that the Library now totals 42,287 volumes and 94,980 reprints, the Librarian is under the obligation to state that the stacks for serials are filled with an allowance of growth space for each current set for the next four years (including 1937) and that the serial sets are now spread through the entire fifth, fourth and second floors and the side shelves of the third (book) floor and the first (reprint) floor. This arrangement and spacing of the serial sets was accomplished during the fall of 1936. Space for new "back sets" can be made at one end of the "book stack" for a few years, since the present book holdings may be crowded into two thirds of the space they now occupy. The reprint boxes fill the first floor space allotted to them and will be very crowded before the end of four years. All duplicate serials and reprints have now been housed outside the Library. During the year many duplicate serials and reprints were sold or exchanged. This is shown in the unusual sales and filled-in serial sets recorded above. It is necessary to explain also how 50,000 volumes, which will be the total in four years if growth occurs at the present rate of about 2,000 annually, and 108,000 reprints if each year adds 3,500, will completely fill space that in 1925 was estimated to be adequate for 100,000 volumes, or 20,000 on each of five floors. The reprint floor at once reduces the available space for volumes to a capacity for 80,000. Besides this the many serial sets and books of quarto size and over reduce the space and half of the bound serials recorded in our count are in reality two volumes bound together, so that the Library will at the end of the year 1940 actually be housing more nearly 75,000 volumes counted as volumes and not by the accession number, and 108,000 reprints.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-ninth session of the Marine Biological Laboratory for the year 1936.

1. *Attendance.* An inspection of the tabular summary of attendance on page 29 of this report and the corresponding summaries in earlier reports shows that during the past 14 years the attendance of investigators at the Marine Biological Laboratory has passed through two distinct phases and now seems to be entering into a third. The first phase was marked by a steady annual increase during which the number of

investigators grew from 176 in 1923 to 362 in 1931, the latter figure being the largest yet reached in the history of the Laboratory. In 1932, when the effects of the depression had become fully felt by most American colleges and universities, the second phase began with a sharp drop in the attendance to 314, at approximately which level it was maintained with no significant changes for four years. In 1936 with a suddenness equalled only by that of the decrease in 1932 a large increase brought the figure back almost to its highest previous level (359 in 1936 as compared with 362 in 1931). Indeed, since the number of students, which fluctuates within the rather narrow limits from year to year, happened to be at about its maximum in 1936, the total attendance of investigators and students together, after allowing for duplications, was in that year the largest in the history of the Laboratory (473 in 1936 as compared with 467 in 1931). Particularly worthy of mention is the very large number of institutions represented by students and investigators in 1936 (158 as compared with the next-highest number of 143 in 1935).

At the time of the writing of this report, though accurate figures are not yet available, it appears likely that the attendance in 1937 will surpass all previous records. If, as seems possible, the Laboratory is now entering into a new period of increasing attendance, serious consideration must be given to the best means of preventing undue crowding in the future. Since without additions to the present laboratory buildings the maximum number of investigators that can be accommodated at one time does not greatly exceed the figures reached in 1931 and in 1936, further increases will be possible only by lengthening the season and flattening the peak of attendance which now occurs early in August. That very considerable possibilities in these directions still exist is shown by the following tabulation of the attendance during the past ten years at approximately ten-day intervals throughout the working season.

		1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
May	30	7	15	9	6	6	8	11	12	11	14
June	10	50	64	55	50	51	54	46	54	43	41
"	20	114	140	139	153	153	127	129	137	127	142
"	30	212	240	197	208	217	172	184	196	174	190
July	10	247	281	238	253	258	225	235	249	226	242
"	20	247	282	242	250	273	245	253	256	232	260
"	30	245	272	249	253	281	248	255	248	257	272
August	10	234	250	256	254	302	257	261	264	245	282
"	20	208	226	243	245	280	236	244	250	235	266
"	30	168	183	220	204	239	190	205	211	192	223
September	10	110	112	157	122	136	129	117	93	94	121
"	20	50	43	59	44	69	58	45	38	26	47
"	30	12	14	14	8	14	13	12	9	11	20

2. *The Report of the Treasurer.* While the figures given above

indicate that as measured by its attendance the recovery of the Laboratory from the effects of the depression is now substantially complete, the same is not yet true with regard to its financial position, which though entirely sound, is still such as considerably to restrict its scientific activities. A study of the reports of the Treasurer for the past 6 years shows that the chief reason for this condition is the reduced income from the Endowment Fund. In 1931 the amount received from this source was $57,728.26; by 1934 the corresponding figure was $46,939.97. While it is gratifying to be able to record an increase in 1935 of approximately $1,000 above the amount received during the preceding year and a further increase of $2,000 in 1936, it is extremely unlikely, because of the low rates of interest at which funds liberated by the maturing of securities can be reinvested, that the pre-depression income from this source can be restored. Fortunately for the Laboratory, special dividends declared by the General Biological Supply House during the past two years have helped to some extent to make good the loss of income from other sources. It is also encouraging to be able to report a small but satisfactory increase in 1936 over 1935 both in the gross sales and in the profits of the Laboratory's own Supply Department.

Next in importance to the income of the Laboratory from its endowment funds is that from the fees paid by institutions and individuals for research space. That financial recovery has in this case tended to lag behind scientific recovery is shown by the fact that whereas the number of investigators in attendance in 1936 was only 3 less than in 1931 the income from the space they occupied was less by nearly $4,000. To this extent, therefore, the burden of the depression has been transferred from colleges and universities to the Marine Biological Laboratory at a time when its own income from other sources has been significantly reduced. It is encouraging to note, however, that during the past year there has been a substantial increase in the amount received by the Laboratory for research space, the figures for the years 1935 and 1936 being $12,470.00 and $14,215.00 respectively.

3. *The Report of the Librarian.* During the year covered by this report the growth of the Library has continued at a very satisfactory rate. Particularly noteworthy are the increases in the number of journals currently received (1,339 in 1936 as compared with 1,271 in 1935), the completion of a considerable number of back sets of journals, and further important additions to the reprint collection. With complete sets of almost all the more important periodicals in Biology and the related fields accessible at all times in the library itself, and with almost 100,000 reprints available for the use of investigators in their own

rooms, it can now be said without exaggeration that the biologists working at the Marine Biological Laboratory enjoy library facilities which are unsurpassed anywhere. The growth of the library since 1926 is more completely summarized in the following table:

	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Serials received currently	628	764	874	985	1,060	1,080	1,126	1,137	1,197	1,271	1,339
Total number of bound volumes .	18,200	22,800	26,500	28,300	31,500	33,800	36,000	37,400	38,600	40,200	42,000
Reprints	38,000	43,000	51,000	59,000	64,000	70,000	76,000	81,000	86,000	92,000	95,000

4. *Courses of Instruction.* At its last meeting in 1936 the Executive Committee received and accepted with regret the resignation of Dr. Elbert C. Cole as head of the Course in Invertebrate Zoölogy, a position which he had filled with conspicuous success since 1932. As his successor the Committee appointed Dr. T. H. Bissonnette of Trinity College, who as a member of the Staff since 1926 and as Acting Head of the Course in 1936 is peculiarly well fitted to continue the work so ably directed by Dr. Cole and his predecessors.

5. *Lectures and Scientific Meetings.* During the past summer 13 evening lectures were given and 9 evening meetings were held for the presentation and discussion of shorter papers by investigators associated with the three Woods Hole scientific institutions. The number of papers so presented was 37; their titles are listed on page 31. In addition, the General Scientific Meeting on August 27 and 28, which was devoted exclusively to work accomplished at the Marine Biological Laboratory during the current season, was the most successful ever held. So large was the number of titles submitted that it was necessary to devote two full mornings to scientific papers and an additional afternoon to demonstrations. The program of this meeting on page 34 and the abstracts of the papers published in the Biological Bulletin for October 1936, give a very excellent picture of the work carried on at the Laboratory during the summer of 1936. In addition to its own scientific activities, the Laboratory also acted as host to the Genetics Society of America, which on September 3, 4 and 5 held in Woods Hole a very well-attended and successful meeting.

6. *Acquisitions of Property.* The past year has seen three important additions to the land and buildings owned by the Marine Biological Laboratory. The first of these, a very generous gift to the Laboratory by Dr. Edward B. Meigs, is the bathing beach and large bathhouse on Buzzard's Bay, together with the remainder of what is officially known as Lot X of the Bay Shore Property of Henry H. and Sarah B. Fay, having a total area of approximately 35,000 square feet. The beach, with a frontage of approximately 200 feet on the water, is the best one

available in the vicinity of the Laboratory, and has been used for many years by our students and investigators, though with some uncertainty as to whether this privilege might at some future time be withdrawn. The generosity of Dr. Meigs has effectively put an end to all such doubts.

A second very important acquisition, obtained through the foresight and generosity of Mr. Charles R. Crane, to whom the Marine Biological Laboratory is already so deeply indebted for support during its early struggles for existence and for very substantial aid in placing it in its present position of security, is that of the land and buildings occupied by the Penzance Garage on Main Street and the Spindell lot adjoining it on the southeast. This tract of over two acres extends from the property owned by the Oceanographic Institution to the entrance into the Eel Pond. Its fine frontage on Great Harbor offers very important possibilities for the future development of the Laboratory.

The third addition, obtained by purchase, is the Howes property on Main Street consisting of a house and barn and a lot 77 by 119 feet lying between the Kidder House and the "Homestead." The acquisition of this property completes the ownership by the Laboratory of the entire block bounded by Main and Center Streets and East and West Streets, respectively. Within these boundaries are the original laboratory building erected in 1888 and most of the wooden buildings added subsequently. The importance to the Laboratory of the possession of this unbroken tract is obvious.

7. *Board of Trustees.* At the annual meeting of the corporation held on Tuesday, August 11, Professor S. O. Mast of Johns Hopkins University was chosen to fill the vacancy in the class of 1940 created by the earlier election at the same meeting of Dr. M. J. Greenman as Trustee Emeritus.

There are appended as parts of the report:

1. The Staff, 1936.
2. Investigators and Students, 1936.
3. A Tabular View of Attendance, 1932–1936.
4. Subscribing and Coöperating Institutions, 1936.
5. Evening Lectures, 1936.
6. Shorter Scientific Papers, 1936.
7. General Scientific Meeting, 1936.
8. Members of the Corporation, August, 1936.

Respectfully submitted,

M. H. JACOBS,
Director.

1. THE STAFF, 1936

Merkel H. Jacobs, *Director,* Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY .

I. Investigation

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
E. G. Conklin, Professor of Zoölogy, Princeton University.
Caswell Grave, Professor of Zoölogy, Washington University.
H. S. Jennings, Professor of Zoölogy, Johns Hopkins University.
Frank R. Lillie, Professor of Embryology Emeritus, The University of Chicago.
C. E. McClung, Professor of Zoölogy, University of Pennsylvania.
S. O. Mast, Professor of Zoölogy, Johns Hopkins University.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoölogy Emeritus, Harvard University.
E. B. Wilson, Professor of Zoölogy Emeritus, Columbia University.
Lorande L. Woodruff, Professor of Protozoölogy, Yale University.

II. Instruction

T. H. Bissonnette, Professor of Biology, Trinity College.
E. C. Cole, Professor of Biology, Williams College. (Absent in 1936.)
C. E. Hadley, Associate Professor of Biology, New Jersey State Teachers College at Montclair.
F. R. Kille, Instructor in Zoölogy, Swarthmore College.
S. A. Matthews, Associate in Anatomy, School of Medicine, University of Pennsylvania.
O. E. Nelsen, Assistant Professor of Zoölogy, University of Pennsylvania.
L. P. Sayles, Assistant Professor of Biology, College of the City of New York.
A. J. Waterman, Assistant Professor of Biology, Williams College.

Junior Instructors

P. S. Crowell, Jr., Instructor in Biology, Brooklyn College.
A. M. Lucas, Associate Professor of Zoölogy, Iowa State College.

PROTOZOÖLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
Elizabeth Drumtra, Instructor in Zoölogy, Wilson College.
G. W. Kidder, Instructor in Zoölogy, College of the City of New York.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

L. G. Barth, Assistant Professor of Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University. (Absent in 1936.)
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Oscar Schotté, Assistant Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Tennessee.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

Robert Chambers, Professor of Biology, New York University.
J. K. W. Ferguson, Assistant Professor of Physiology, University of Western Ontario.
Rudolf Höber, Visiting Professor of Physiology, University of Pennsylvania.
Laurence Irving, Professor of Experimental Biology, University of Toronto.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.
C. Ladd Prosser, Assistant Professor of Physiology, Clark University.

Junior Instructors

Kenneth Fisher, Demonstrator in Biology, University of Toronto.
F. J. M. Sichel, Royal Society of Canada Fellow.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Michigan.
Francis Drouet, Research Fellow, University of Missouri.
G. W. Prescott, Assistant Professor of Biology, Albion College.

GENERAL OFFICE

F. M. MacNaught, Business Manager.
Polly L. Crowell, Assistant.
Edith Billings, Secretary.

RESEARCH SERVICE AND GENERAL MAINTENANCE

Samuel E. Pond, Technical Manager.
Oscar W. Richards, Chemical Service.
G. Failla, X-Ray Physicist.
Thomas E. Larkin, Superintendent.
William Hemenway, Carpenter.
Lester F. Boss, Research Technician.
J. D. Graham, Glassblower.
P. H. Liljestrand, Assistant.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Doris Endrejat, Mary A. Rohan, Assistants.

SUPPLY DEPARTMENT

James McInnis, Manager.
Milton B. Gray, Collector.
A. M. Hilton, Collector.
A. W. Leathers, Shipping Department.
Geoffrey Lehy, Collector.
Walter Kahler, Collector.
Ruth S. Crowell, Secretary.
Anna N. Hall, Secretary.

MUSEUM

George M. Gray, Curator Emeritus.

2. INVESTIGATORS AND STUDENTS, 1936

Independent Investigators

Abramowitz, A. A., Research Assistant, Harvard University.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, W. C., Professor of Zoölogy, The University of Chicago.
Amberson, William R., Professor of Physiology, University of Tennessee.
Anderson, Rubert S., Research Associate, Princeton University.
Appel, F. W., Associate Professor of Biology, St. John's College.
Armstrong, Philip B., Assistant Professor of Anatomy, Cornell University Medical College.
Bailey, Percy L., Jr., Instructor in Physiology, College of the City of New York.
Ball, Eric G., Associate in Physiological Chemistry, Johns Hopkins University Medical School.

Ballard, William W., Assistant Professor, Dartmouth College.
Barth, L. G., Assistant Professor of Zoölogy, Columbia University.
Bauer, Hans G. E., International Research Fellow, Rockefeller Foundation.
Bernstein, Felix, Professor of Biometrics, New York University.
Bissonnette, T. H., Professor of Biology, Trinity College.
Bodine, J. H., Head of Zoölogy Department, University of Iowa.
Bozler, Emil, Fellow in Medical Physics, Johnson Foundation, University of Pennsylvania.
Bray, Charles W., Assistant Professor, Princeton University.
Brinley, F. J., Assistant Professor of Zoölogy, North Dakota State College.
Bronk, D. W., Professor of Biophysics, University of Pennsylvania.
Brooks, M. M., Research Associate in Biology, University of California.
Brooks, S. C., Professor of Zoölogy, University of California.
Brown, Dugald E. S., Assistant Professor of Physiology, New York University, College of Medicine.
Budington, R. A., Professor of Zoölogy, Oberlin College.
Burton, Alan C., Training Fellowship, General Education Board.
Cable, R. M., Assistant Professor of Parasitology, Purdue University.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Cameron, John A., Instructor in Zoölogy, University of Missouri.
Cannan, Robert K., Professor of Chemistry, New York University, College of Medicine.
Carlson, J. G., Instructor in Zoölogy, University of Alabama.
Carothers, E. Eleanor, Research Associate, University of Iowa.
Cattell, McKeen, Associate Professor of Pharmacology, Cornell University Medical College.
Chambers, Robert, Research Professor of Biology, New York University.
Cheney, Ralph H., Professor of Biology, Long Island University.
Child, George P., Research Assistant in Biology, Amherst College.
Clark, Eleanor L., University of Pennsylvania Medical School.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania.
Clement, Anthony C., Assistant Professor of Biology, College of Charleston.
Clowes, G. H. A., Director of Research, Eli Lilly & Co.
Coe, W. R., Professor of Biology, Yale University.
Coker, R. E., Professor of Zoölogy, University of North Carolina.
Coonfield, B. R., Assistant Professor, Brooklyn College.
Copeland, Manton, Professor of Biology, Bowdoin College.
Corey, H. Irene, Research Assistant, University of Pennsylvania.
Co Tui, Frank W., Associate Professor, New York University, College of Medicine.
Cowdry, E. V., Professor of Cytology, Washington University.
Crowell, Prince Sears, Jr., Instructor, Brooklyn College.
Curtis, W. C., Professor of Zoölogy, University of Missouri.
Curwen, Alice O., Assistant Professor of Anatomy, Woman's Medical College of Pennsylvania.
Dan, Katsuma, Research Associate, Misaki Marine Biological Station, Misaki, Japan.
Dildine, Glenn C., Instructor in Zoölogy, Northwestern University.
Diller, William F., Instructor in Zoölogy, Dartmouth College.
Donaldson, Henry H., Member, Wistar Institute.
Dornfeld, Ernst J., Assistant in Zoölogy, University of Wisconsin.
Dreyer, William A., Instructor in Zoölogy, University of Cincinnati.
Drouet, Francis, Research Fellow, University of Missouri.
duBuy, H., Research Fellow, Harvard Medical School.
Dunihue, F. W., Instructor, New York University.
Ets, Harold N., Associate Professor, Loyola University, School of Medicine.

Ferguson, James K. W., Assistant Professor of Physiology, University of Western Ontario, Medical School.
Figge, Frank H. J., Associate Professor of Anatomy, University of Maryland Medical School.
Fleisher, Moyer S., Professor of Bacteriology and Hygiene, St. Louis University.
French, C. S., 18 Tremont Street, Boston, Massachusetts.
Fry, Henry J., Visiting Investigator, Cornell University Medical College.
Garrey, W. E., Professor of Physiology, Medical School, Vanderbilt University.
Gerard, Ralph W., Associate Professor of Physiology, The University of Chicago.
Gilchrist, Francis G., Assistant Professor of Zoölogy, Pomona College.
Goldfelder, Anna, Research Assistant, Columbia University, Institute of Cancer Research.
Gottschall, Gertrude G., Assistant in Biochemistry, Cornell University Medical College.
Grave, B. H., Professor of Zoölogy, DePauw University.
Grave, Caswell, Professor of Zoölogy, Washington University.
Hadley, Charles E., Associate Professor of Biology, New Jersey State Teachers College.
Hartline, H. K., Fellow in Medical Physics, University of Pennsylvania.
Harvey, Ethel Browne, Princeton University.
Headlee, William H., Instructor in Biology, Purdue University.
Heilbrunn, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
Henshaw, Paul S., Biophysicist, Memorial Hospital.
Hess, Walter N., Head of Biology Department, Hamilton College.
Hibbard, Hope, Associate Professor of Zoölogy, Oberlin College.
Hill, Samuel E., Assistant in General Physiology, Rockefeller Institute.
Hoadley, Leigh, Professor of Zoölogy, Harvard University.
Höber, Josephine, University of Pennsylvania.
Höber, Rudolf, Visiting Professor of Physiology, University of Pennsylvania.
Hollaender, Alexander, Special Investigator, Radiation Committee, National Research Council, University of Wisconsin.
Holtfreter, Johannes, Assistant, Zoologisches Institut, München, Germany.
Hopkins, Dwight L., Assistant Professor of Zoölogy, Duke University.
Hörstadius, Sven, Associate Professor, University of Stockholm, Stockholm, Sweden.
Howe, H. E., Editor, Industrial and Engineering Chemistry.
Hummel, Katharine P., Research Instructor, Cornell University.
Irving, Laurence, Professor of Experimental Biology, University of Toronto.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Jenkins, George B., Professor of Anatomy, Medical School, George Washington University.
Johlin, J. M., Associate Professor of Biochemistry, Vanderbilt University Medical School.
Jones, E. Ruffin, Jr., Associate Professor, College of William and Mary.
Jones, Ruth McC., Instructor in Zoölogy and Botany, Swarthmore College.
Keil, Elsa M., Assistant Professor of Zoölogy, Rutgers University.
Keltch, Anna K., Research Chemist, Eli Lilly & Co.
Kidder, George W., Instructor, College of the City of New York.
Kille, Frank R., Instructor, Swarthmore College.
Kindred, James E., Associate Professor of Histology and Embryology, University of Virginia, Medical School.
King, Jessie L., Professor of Physiology, Goucher College.
Kleinholz, L. H., Austin Teaching Fellow, Harvard University.
Knisely, Melvin H., Fellow, General Education Board.
Knower, Henry McE., Research Associate in Biology, Yale University.

Knowlton, Frank P., Professor of Physiology, Syracuse University, Medical College.
Kopac, M. J., Research Associate in Biology, New York University.
Korr, Irvin M., Procter Fellow, Princeton University.
Kraatz, C. P., Graduate Assistant in Zoölogy, University of Cincinnati.
Krahl, M. E., Research Chemist, Eli Lilly & Co.
Kuttner, Ann G., Resident Physician, Rockefeller Institute.
Lancefield, Donald E., Professor of Zoölogy, Columbia University.
Lancefield, Rebecca C., Associate in Bacteriology, Rockefeller Institute.
Lehmann, Jörgen, Rockefeller Fellow, Rockefeller Institute for Medical Research.
Levine, Darwin S., Teacher of Biology, Theodore Roosevelt High School, New York City, New York.
Lillie, Frank R., Professor of Embryology, Emeritus, The University of Chicago.
Lillie, Ralph S., Professor of General Physiology, The University of Chicago.
Lindeman, V. F., Assistant Professor of Zoölogy, Syracuse University.
Loomis, W. E., Associate Professor of Plant Physiology, Iowa State College.
Lucas, Alfred M., Associate Professor of Zoölogy, Iowa State College.
Lucas, Miriam Scott, 412 Tenth Street, Ames, Iowa.
Lucké, Balduin, Professor of Pathology, University of Pennsylvania, School of Medicine.
Luyet, Basile J., Associate Professor of Biology, St. Louis University.
Lynn, W. Gardner, Instructor, Johns Hopkins University.
MacCardle, Ross C., Assistant Professor of Biology, Temple University.
MacDougall, Mary Stuart, Professor of Zoölogy, Agnes Scott College.
Maclean, Bernice L., Instructor in Zoölogy, Hunter College.
Magers, Elizabeth, Assistant Professor of Physiology, Vassar College.
Magruder, Samuel R., Research Associate, University of Cincinnati.
Marsland, Douglas A., Assistant Professor of Biology, Washington Square College, New York University.
Martin, Earl A., Professor of Biology, Brooklyn College.
Martin, Walter E., Instructor, Purdue University.
Mast, S. O., Department of Zoölogy, Johns Hopkins University.
Mathews, Albert P., Professor of Biochemistry, University of Cincinnati.
Matthews, Samuel A., Associate in Anatomy, University of Pennsylvania.
Mavor, James W., Professor of Biology, Union College.
McClung, C. E., Director, Department of Zoölogy, University of Pennsylvania.
McGregor, James H., Professor of Zoölogy, Columbia University.
Michaelis, Leonor, Member, Rockefeller Institute for Medical Research.
Miller, Forrest W., Research Entomologist, Amherst College.
Moment, Gairdner B., Instructor, Goucher College.
Morgan, Lilian V., Pasadena, California.
Morgan, T. H., Professor of Experimental Zoölogy, California Institute of Technology.
Morrill, Dr. C. V., Cornell University Medical College.
Muller, H. J., Geneticist, Institute of Genetics, Moscow, Russia.
Nabrit, S. M., Professor of Biology, Atlanta University.
Nahm, Laura J., Instructor in Biology, Flat River Junior College.
Navez, Albert E., Milton Academy.
Nelsen, Olin E., Assistant Professor of Zoölogy, University of Pennsylvania.
Nonidez, José F., Assistant Professor of Anatomy, Cornell University Medical College.
Northrop, John H., Member, Rockefeller Institute.
Nunnemacher, Rudolph F., Assistant, Harvard University.
Orr, Paul R., Instructor in Biology, Brooklyn College.

Osterhout, W. J. V., Member, Rockefeller Institute for Medical Research.
Packard, Charles, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Painter, Elizabeth E., Instructor in Physiology, University of Maryland, School of Medicine.
Parker, G. H., Professor of Zoölogy, Emeritus, Harvard University.
Parpart, Arthur K., Assistant Professor, Princeton University.
Plough, Harold H., Professor of Biology, Amherst College.
Pond, Samuel E., Technical Manager, Marine Biological Laboratory.
Prescott, G. W., Assistant Professor of Biology, Albion College.
Prosser, C. Ladd, Assistant Professor of Physiology, Clark University.
Puckett, William O., Instructor in Biology, Princeton University.
Raffel, Daniel, Research Geneticist, Institute of Genetics, Moscow, Russia.
Rice, Kenneth S., Woods Hole, Massachusetts.
Richards, Oscar W., Instructor in Biology, Yale University.
Robertson, C. W., Assistant in Biology, Washington Square College, New York University.
Root, Walter S., Associate Professor of Physiology, Medical School, University of Maryland.
Rugh, Roberts, Instructor in Zoölogy, Hunter College.
Sabin, Albert B., Assistant on the Scientific Staff, The Rockefeller Institute for Medical Research.
Sandow, Alexander, Instructor, Washington Square College.
Saslow, George, Assistant Professor of Biology, New York University.
Sayles, Leonard P., Assistant Professor of Biology, College of the City of New York.
Schechter, Victor, Instructor, College of the City of New York.
Schenthal, Joseph E., Julius Friedenwald Research Fellow, University of Maryland, Medical School.
Schmidt, L. H., Research Fellow, Christ Hospital.
Schmitt, Francis O., Associate Professor of Zoölogy, Washington University.
Schotté, Oscar E., Assistant Professor of Biology, Amherst College.
Schrader, Franz, Professor of Zoölogy, Columbia University.
Schrader, Sally Hughes, Member, Natural Science Faculty, Sarah Lawrence College.
Schwab, Joseph J., Assistant in Zoölogy, The University of Chicago.
Scott, Allan C., Instructor in Biology, Union College.
Scott, J. Paul, Associate Professor of Zoölogy, Wabash College.
Shapiro, Herbert, Research Assistant, Princeton University.
Sichel, Ferdinand J. M., Fellowship, Royal Society of Canada.
Sickles, Gretchen R., Senior Laboratory Technician, New York State Department of Health.
Smelser, George K., Instructor in Anatomy, Columbia University.
Smith, Dietrich C., Instructor in Physiology, University of Tennessee.
Smith, H. P., Professor of Pathology, University of Iowa.
Speicher, B. R., National Research Fellow, Columbia University.
Speicher, Kathryn G., New York City, New York.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Stein, Kathryn F., Assistant Professor, Mount Holyoke College.
Steinbach, H. Burr, Instructor in Zoölogy, University of Minnesota.
Stern, Kurt G., Visiting Lecturer in Physiological Chemistry, Yale University.
Stewart, Dorothy R., Assistant Professor of Biology, Skidmore College.
Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.

Strong, Oliver S., Professor of Neurology and Neuro-histology, Columbia University.
Stunkard, H. W., Professor of Biology, New York University.
Summers, Francis M., Instructor in Biology, Bard College, Columbia University.
Szepsenwol, Josel, University of Geneva.
Taft, Charles H., Jr., Associate Professor of Pharmacology, Medical Branch, University of Texas.
Tashiro, Shiro, Professor of Biochemistry, University of Cincinnati, Medical College.
Taylor, William Randolph, Professor of Botany, University of Michigan.
TeWinkel, Lois E., Assistant Professor of Zoölogy, Smith College.
Tracy, Henry C., Anatomy Department, University of Kansas.
Trager, William, Assistant, Rockefeller Institute.
Uhlenhuth, Eduard, Professor of Anatomy, University of Maryland, Medical College.
Varrelman, Ferdinand, American University.
Walker, Roland, Instructor in Biology, Rensselaer Polytechnic Institute.
Warner, E. O., Assistant Professor of Pathology, University of Iowa.
Warren, Marshall R., Graduate Assistant in Zoölogy, University of Cincinnati.
Waterman, A. J., Assistant Professor of Biology, Williams College.
Weissenberg, Richard, University of Berlin.
Wells, G. P., Lecturer, University College, London, England.
Whedon, Arthur D., Professor of Zoölogy and Head of Department, North Dakota State College.
Whitaker, D. M., Professor of Biology, Stanford University.
Willier, B. H., Professor and Head of Department of Zoölogy, University of Rochester.
Wilson, Edmund B., Professor Emeritus in Residence, Columbia University.
Wintrobe, Maxwell M., Associate in Medicine, Johns Hopkins University.
Wolf, E. Alfred, Associate Professor of Biology, University of Pittsburgh.
Wolf, Ernst, Research Associate, Harvard University.
Wolf, Opal M., Lecturer, Barnard College.
Woodruff, Lorande L., Professor of Protozoölogy, Yale University.
Yntema, C. L., Instructor, Cornell University Medical College.
Young, John Z., Fellow, Magdalen College, Oxford, England.
Young, Roger A., Assistant Professor of Zoölogy, Howard University.
Youngstrom, Karl A., Instructor in Anatomy, Kansas University.

Beginning Investigators

Albaum, Harry G., Graduate Student, Columbia University.
Algire, Glenn H., Graduate Student, University of Maryland, School of Medicine.
Andersch, Marie, Assistant Professor of Biochemistry, Woman's Medical College, Philadelphia, Pennsylvania.
Angerer, C. A., Instructor, University of Pennsylvania.
Arthur, J. B. M., Jr., Laboratory Assistant, Amherst College.
Bishop, David W., Instructor, University of Pennsylvania.
Bocskey, Stephen C., Associate Professor of Biology, University of Notre Dame.
Brooks, Jeanne R., Oberlin College.
Chambers, Alfred H., Jr., Swarthmore College.
Churney, Leon, Instructor in Zoölogy, University of Pennsylvania.
Compton, Alfred D., Jr., Graduate Student, Yale University.
Copeland, D. Eugene, Biology Assistant, Amherst College.
Corson, Samuel A., Research Associate, Washington Square College, New York University.
Dan, Jean Clark, University of Pennsylvania.

Denny, Martha, Assistant in Zoölogy, Barnard College.
Donnellon, James A., University of Pennsylvania.
Dordick, Isadore, Graduate Student, University of Pennsylvania.
Drumtra, Elizabeth, Instructor, Wilson College.
Dugal, Louis-Paul, Instructor in Biology, University of Montreal.
Emerson, Henry S., Graduate Assistant, Amherst College.
Fisher, Kenneth C., Demonstrator in Biology, University of Toronto.
Forman, Richard C., Graduate Student, Amherst College.
Friedman, Sam, Graduate Student, Washington Square College, New York University.
Fronczak, Michael I., Assistant Advisor to the Department of Biology, Seton Hall College.
Greenfield, Sydney S., Graduate Student, Brooklyn College.
Halbreich, Morton A., Graduate Student, University of Pennsylvania.
Harris, Daniel L., University of Pennsylvania.
Hawley, Katharine J., Smith College.
Henson, Margaret, Smith College.
Hershkowitz, Sol. G., Student, New York University, College of Medicine.
Hirschfeld, Nathan B., Student, Columbia University.
Hollingsworth, Josephine, University of Pennsylvania.
Hornor, Helen B., Assistant in Zoölogy, Barnard College, Columbia University.
Horton, R. G., Graduate Student, Cornell University.
Hughes, Roscoe D., Assistant in Zoölogy, Columbia University.
Hunninen, Arne V., Assistant, Johns Hopkins University.
Hunter, Francis R., Part-time Teaching Assistant, Princeton University.
Hunter, Laura N., Graduate Student, University of Pennsylvania.
Hutchings, Lois M., Teacher of Biology, Weequahic High School.
Itoh, Hidegoro, Graduate Student, Zoölogical Laboratory, University of Pennsylvania.
Jailer, Joseph W., Teaching Fellow, Washington Square College, New York University.
Jakobsen, Edith M., Science Teacher, Hawthorne High School, Hawthorne, New Jersey.
Kaliss, Nathan, Assistant in Zoölogy, Columbia University.
Kaufman, Alan L., Student, Franklin and Marshall College.
Kehoe, Catharine E., Research Assistant, Oberlin College.
Lilly, Daniel M., Instructor in Biology, Providence College.
Lipman, Harry J., Graduate Assistant, University of Pittsburgh.
Long, Margaret E., Graduate Student, University of Pennsylvania.
Lord, Robert N., Student, Williams College.
Marquette, William G., Graduate Student, Columbia University.
Mayo, Virginia, Teacher, Chairman, Department of Science, Dana Hall School.
Mazia, Daniel, Harrison Fellow in Zoölogy, University of Pennsylvania.
McBride, T. F., Instructor Clinical Dentistry, School of Dentistry, University of Pittsburgh.
Mendoza, Guillermo, Teaching Assistant, Northwestern University.
Moser, Floyd, Graduate Student, University of Pennsylvania.
Newman, Morris, Graduate Student, University of Pennsylvania.
Novikoff, Alex B., Tutor, Brooklyn College.
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Olson, Rodney A., Tufts College.
Palmer, Charles M., Assistant Professor of Botany, Butler University.
Peabody, Elizabeth B., Graduate Student, Radcliffe College.
Richardson, Estelle, Graduate Student, New York University.
Rose, S. Meryl, Assistant, Columbia University.

ROSENFIELD, RICHARD E., Graduate Student, University of Pittsburgh.
SCHOENBORN, HENRY W., Graduate Assistant, New York University.
SCHOEPFLE, GORDON M., Undergraduate Assistant, DePauw University.
SCOTT, BIRDIE L., Student and Graduate Assistant, Atlanta University and Spelman College.
SEITCHEK, JOSEPH N., Graduate Student, University of Pennsylvania.
SOLBERG, ARCHIE N., University Fellow, Columbia University.
SPECHT, HEINZ, Assistant in Physiology, New York University College of Medicine.
STOCK, CHARLES C., Graduate Student in Physiological Chemistry, Johns Hopkins Medical School.
TEITELBAUM, HARRY A., Instructor in Anatomy, University of Maryland, Medical School.
WALBORN, REBECCA S., Graduate Assistant in Zoölogy, University of Missouri.
WHEELER, NORMAN C., Assistant in Physiology, Purdue University.
WICHTERMAN, RALPH, Graduate Student, University of Pennsylvania.

Research Assistants

ARMSTRONG, LOUISE S., Research Assistant, Cornell University Medical College.
ATCHLEY, DANA W., JR., Student, Harvard College.
BEAR, RICHARD S., Research Assistant, Washington University.
BECK, LYLE V., Varr Cott Research Fellow, Long Island College of Medicine.
BOSWORTH, MILLARD W., Research Assistant, Wesleyan University.
BUCHHEIT, J. ROBERT, 1676 Van Buren Street, Saint Paul, Minnesota.
CARMICHAEL, J. C., Student, Vanderbilt University, Medical School.
CASTLE, RUTH M., New Jersey State Teachers College.
CLARK, JOHN K., Assistant, Trinity College.
EMBDEN, MAJA, Research Assistant, University of Toronto.
ERLANGER, MARGARET, Agnes Irwin School.
EVANS, GERTRUDE, Research Assistant, The University of Chicago.
FENNELL, R. A., Research Assistant, Johns Hopkins University.
FIGGE, ROSALIE YERKES, University of Maryland, Medical School.
FLYNN, CARL M., Instructor, University of Maine.
FOLCH-PI, JORDI, Research Fellow, Rockefeller Institute.
FRANKENSTEIN, NORMAN L., Marquette University.
GLASSMAN, HAROLD N., Graduate Student, University of Pennsylvania.
GODRICH, JACK, Research Assistant, Columbia University.
GOFFIN, CATHERINE E., Research Assistant, Eli Lilly & Co.
GOLDIN, ABRAHAM, Laboratory Assistant, Brooklyn College.
GREEY, CONSTANCE M., University of Toronto.
GREEY, ELIZABETH L., Research Assistant, University of Toronto.
HILL, EDGAR S., Research Assistant, Washington University.
HOBSON, LAWRENCE B., Graduate Assistant in Zoölogy, University of Cincinnati.
KAYLOR, CORNELIUS T., Fellow in Biology, Princeton University.
LEDERMAN, EDWARD, Assistant, University of Cincinnati.
LEVIN, LOUIS, Laboratory Assistant, DePauw University.
MARMER, DINA, Assistant, Columbia University.
MAST, ELISABETH T., Research Assistant in Psychology, Johns Hopkins University.
MILLS, KATHARINE O., Technician and Assistant, University of Missouri.
MONKE, J. VICTOR, Teaching Fellow, University of Tennessee, Medical School.
MOORE, JOHN A., Assistant in Zoölogy, Columbia University.
MORAQUEZ, V., Barcelona Medical School, Spain.
MORRIS, MARION C., Washington University.
NESTLER, HERBERT A., Reader, Brooklyn College.

Nicoll, Paul A., Washington University.
O'Brien, Helen, University of Pennsylvania.
Pappenheimer, John R., Research Assistant, Harvard University.
Reeder, Elizabeth M., Instructor in Zoölogy, University of Missouri.
Ricca, Renato A., Research Assistant, University of Pennsylvania.
Robertson, Kay, Research Fellow, University of Toronto.
Robertson, Lola E., Research Assistant, New York University.
Robinson, R. A., University of Pennsylvania.
Rose, Earl T., Milford, Iowa.
Rubin, Solomon H., Teaching Fellow, New York University College of Medicine.
Salk, Jonas, Fellow in Chemistry, New York University.
Shaw, Isidor, Research Assistant, Long Island University.
Smith, Jay A., Assistant in Department of Zoölogy, DePauw University.
Stanbury, John, Student, Harvard Medical School.
Steiman, S. E., Boston University.
Taylor, John F., Johns Hopkins Medical School.
Thompson, James U., Weaver Fellow in Anatomy, University of Maryland, Medical School.
Thornton, C. S., Assistant, Princeton University.
Weisberg, Harry F., Fellow in Vertebrate Zoölogy, College of the City of New York.
Wightman, John C., Assistant, Brown University.
Young, S. B., Technician, Rockefeller Institute for Medical Research.

Students

BOTANY

Ashton, Miriam R., Instructor, University of British Columbia.
Giles, George H., High School Teacher, Wilsonville, Nebraska.
Palmer, Charles M., Graduate Student, Indiana University.
Poole, Margery, Student, Radcliffe College.
Scheer, Beatrice A., Assistant in Botany, Connecticut College.
Shurtleff, Rosamond L., Student, Wheaton College.
Thurlow, Martha, Goucher College.
Trombetta, Vivian V., Assistant in Botany, Barnard College, Columbia University.
Velasquez, Gregorio T., Graduate Student, University of Michigan.
Williams, Jean L., Botany Assistant, Wellesley College.

EMBRYOLOGY

Ades, Harlow W., Assistant, Department of Zoölogy, University of Illinois.
Arthur, James B. McK., Jr., Student, Amherst College.
Bauer, Donald, Student, Dartmouth College.
Bloch, Janet, Student, Sarah Lawrence College.
Brooks, Jeanne R., Oberlin College.
Clark, Beatrice, Wellesley College.
Colie, Elizabeth S., 377 Voss Avenue, South Orange, New Jersey.
Conant, Betsy D., Graduate Student, University of Rochester.
Culberson, Mabel H., Instructor, Simmons College.
Danner, Edwin C., Assistant Instructor, University of Illinois.
Forbes, Thomas R., Fellow in Anatomy, University of Rochester.
Fowler, Ward S., Student, Swarthmore College.
Froelich, Helen L., Science Librarian, Stephens College.

Fronczak, Michael I., Department Adviser, Seton Hall College.
Gayer, H. Kenneth, Oberlin College.
Henson, Margaret, Student, Smith College.
Hummel, Katharine P., Instructor in Zoölogy, Mount Holyoke College.
Johnson, David F., Graduate Student, New York University.
Joslin, Stuart L., Wesleyan University.
Kennedy, Kathleen M., Demonstrator, Memorial University College.
Klamer, Charles H., Student, Wabash College.
Kriete, Bertrand C., DePauw University.
Lipman, Harry J., Graduate Assistant, University of Pittsburgh.
McCarrell, Jane D., Instructor, Vassar College.
Mendoza, Guillermo, Graduate Assistant, Northwestern University.
Morgan, Theodore J., Fellow in Biology, Washington and Jefferson College.
Pack, Virginia L., Student, Sarah Lawrence College.
Price, John W., Associate Professor of Zoölogy, Ohio State University.
Reddick, Mary L., Assistant Teacher, Spelman College.
Russell, Alice M., Instructor, University of Pennsylvania.
Schneider, Ruth M., Student, Skidmore College.
Smith, Gordon L., Undergraduate Assistant, DePauw University.
Shykin, Pearl, Student, Radcliffe College.
Walborn, Rebecca S., Assistant in Zoölogy, University of Missouri.

PHYSIOLOGY

Andersch, Marie A., Assistant Professor of Biochemistry, Woman's Medical College, Philadelphia, Pennsylvania.
Ballentine, Robert G., Princeton University.
Cameron, John A. C., Instructor in Zoölogy, University of Missouri.
Chambers, Alfred H., Jr., Swarthmore College.
Dugal, Louis-Paul, Instructor in Biology, University of Montreal.
Goldwasser, Seymore, Amherst College.
Grossman, Jacob, Graduate Student, Columbia University.
Lewis, Lena A., Technician, Lancaster General Hospital.
Lilly, Daniel McQ., Instructor in Biology, Providence College.
Magalhaes, Hulda, Graduate Assistant, Mount Holyoke College.
Magers, Elizabeth, Assistant Professor of Physiology, Vassar College.
Margolis, Fred, Student, University of Pittsburgh.
McDonald, Margaret R., Graduate Student, Rutgers University.
Mills, Katharine O., Technician and Assistant, University of Missouri.
Moraquez, Vicens, Facultad de Medicina, Barcelona, Spain.
Ratnoff, Oscar D., Student, College of Physicians and Surgeons.
Reed, Emerson A., Graduate Student and Teaching Assistant, University of California.
Richardson, Estelle D., Graduate Student, New York University.
Rosenfield, Richard E., Student, University of Pittsburgh.
Smith, Judith D., Laboratory Assistant in Physiology, Wellesley College.
Smith, Paul E., Graduate Assistant, University of Rochester.
Wood, Alberta Brown, 75 East 55th Street, New York City, New York.

PROTOZOOLOGY

Berenberg, Naomi R., Student, New Jersey College for Women.
Bocskey, Stephen C., Associate Professor of Biology, University of Notre Dame.
Brush, Ruth M., Hunter College.

Bush, Aeleta N., 723 Williams Street, Atlanta, Georgia.
Claff, C. Lloyd, 5 Van Beal Road, Randolph, Massachusetts.
Cunningham, Katherine, American University.
Dewey, Virginia C., Technician, Harvard Medical School.
Ferenbach, Carl, Student, Princeton University.
Greenfield, Sydney S., Student, Brooklyn College.
Grossman, Cecelia M., Teacher of Biology, Abraham Lincoln High School.
Hudson, Grace P., Hunter College.
Lee, George O., Columbia University.
Lewis, Wilma M., Student Assistant, State Teachers College, Montclair.
Meglitsch, Paul A., Student, University of Illinois.
Metcalf, Isaac S. H., Assistant in Zoölogy, Columbia University.
Mitchell, Alison M., Student, New Jersey College for Women.
Mock, Sarah H., Graduate Student, Missouri University.

INVERTEBRATE ZOÖLOGY

Allen, Thomas H., University of Iowa.
Babcock, Ruth H., Teacher, Caldwell High School, New Jersey.
Bader, Joan E., Student, Montclair State Teachers College.
Bishop, David W., Instructor, University of Pennsylvania.
Bonnet, David D., Harvard University.
Bowen, William J., Johns Hopkins University.
Burlington, Mary, McGill University.
Bush, Aeleta N., 723 Williams Street, Atlanta, Georgia.
Carson, Hampton L., Jr., University of Pennsylvania.
Cassidy, Morton H., Instructor in Biology, Hyde Park High School.
Caylor, Richard L., Associate Professor of Biology, Delta State Teachers College.
Copeland, Donald E., Graduate Assistant, Amherst College.
Cregan, Sister Mary Bertha, Professor of Biology, St. Xavier College.
Croasdale, Hannah T., Research Assistant, Dartmouth College.
Dawson, Ralph W., Assistant Professor, University of Minnesota.
Doyle, Winfield G., Oberlin College.
Faben, Ann Reed, Goucher College.
Farraday, Clayton L., Jr., Swarthmore College.
Granger, Barbara S., Research Assistant and Graduate Student, Mount Holyoke College.
Grave, Caswell, II, Assistant, Washington University.
Grobstein, Clifford, College of the City of New York.
Harris, W. Alfred, DePauw University.
Hill, David L., Graduate Student, State University of Iowa.
Hogan, Sister Stella Maria, Professor of Biology, St. Xavier College.
Hoyt, J. Southgate Y., Washington and Lee University.
Huntington, Margaret O., Swarthmore College.
Johnson, Reuben B., Jr., Connecticut State College.
Kimball, Richard F., Student, Johns Hopkins University.
Knoth, Sibyl C., Head of Science Department, Gulf Park College.
Koster, Rudolf, Graduate Student, Harvard University.
Lewis, Wilma M., Student Assistant, New Jersey State Teachers College.
Morgan, Gwendolynn W., Sarah Lawrence College.
Moyer, Elizabeth K., Graduate Assistant, Mount Holyoke College.
Norris, Charles H., Hamilton College.
Potts, Hugh E., New York University.
Ray, David T., Teacher, Johnson C. Smith University.

Reed, Mary V., Instructor in Science and Mathematics, The Knox School.
Roxby, John B., Jr., Graduate Student, Wesleyan University.
Sarin, Leon, Student, Colby College.
Saslow, George, Assistant Professor of Biology, New York University.
Seaton, Mary J., Pennsylvania College for Women.
Sensenig, Wayne, Jr., Student, Haverford College.
Shelton, Meredith, Sarah Lawrence College.
Spratt, Nelson T., Jr., Instructor in Biology, Emory University.
Stevenson, James H., Assistant in Zoölogy, Oberlin College.
Stokes, Miriam, Graduate Assistant, Mount Holyoke College.
Stump, Alexander B., Student, University of Virginia.
Swift, Katharine W., Student, Smith College.
Twichell, Allen R., Student, Wabash College.
Waterman, Talbot H., Harvard University.
Weierbach, Lily A., University of Pennsylvania.
Weinberg, Stanley L., Graduate Student, Columbia University.
Weir, Ellen H., Student, Wilson College.
Wheeler, Norman C., Assistant in Physiology, Purdue University.
Wood, Elizabeth C., Montclair State Teachers College.

3. TABULAR VIEW OF ATTENDANCE

	1932	1933	1934	1935	1936
Investigators—Total	314	319	323	315	359
Independent	212	210	222	208	226
Under Instruction	73	66	49	56	76
Research Assistants	29	43	52	51	57
Students—Total	132	118	131	130	138
Zoölogy	55	54	54	55	55
Protozoölogy	16	11	11	16	17
Embryology	29	28	30	33	34
Physiology	18	19	23	20	22
Botany	14	6	13	6	10
Total Attendance	446	437	454	445	497
Less Persons registered as both students and investigators	14	12	15	16	24
	432	425	439	429	473
Institutions Represented—Total	141	120	131	143	158
By Investigators	94	92	98	111	120
By Students	76	58	75	70	77
Schools and Academies Represented					
By Investigators	–	1	1	–	2
By Students	1	2	5	3	3
Foreign Institutions Represented					
By Investigators	8	5	4	7	9
By Students	1	–	1	1	5

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1936

American University
Amherst College
Atlanta University
Bowdoin College
Bryn Mawr College
College of Charleston
Columbia University
Cornell University
Cornell University Medical College
DePauw University
Duke University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Iowa State College
Johns Hopkins University
Johnson Foundation
Eli Lilly & Co.
Long Island University
Memorial Hospital, New York City
Morehouse College
Mount Holyoke College
New York State Department of Health
New York University
New York University Medical School
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Purdue University
Radcliffe College
Rensselaer Polytechnic Institute
Rockefeller Foundation
Rockefeller Institute for Medical Research
St. Xavier College
Seton Hall College
Smith College
Swarthmore College
Syracuse University
Temple University
Tufts College
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Iowa
University of Kansas
University of Maryland Medical School
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Wellesley College
Wesleyan University
Wheaton College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1936

Friday, June 26
Dr. A. C. Redfield .... "The Ecological Significance of the Circulation of the Gulf of Maine."

Friday, July 3
Dr. H. K. Hartline .... "Electrical Studies of Visual Mechanisms."

Friday, July 10	Dr. W. C. Allee	“Recent Studies in Mass Physiology.”
Friday, July 17	Dr. G. H. Parker	“Neurohumors as the Means of Animal Color Changes.”
Friday, July 24	Dr. C. E. Renn	“Problems of the Eel Grass Situation.”
Friday, July 31	Dr. Sven Hörstadius	“Researches on Determination in the Early Development of the Sea-urchin.”
Friday, August 7	Dr. J. K. W. Ferguson	“Newer Views of CO_2 Transport and Their Significance to Other Physiological Processes.”
Friday, August 14	Dr. H. W. Stunkard	“Life Cycles of Digenetic Trematodes.”
Wednesday, August 19	Dr. J. R. Katz	“Submicroscopical Structure of Living Organs (Muscle, etc.) Revealed by X-Rays.”
Friday, August 21	Mr. J. Z. Young	“Giant Nerve Fibres in the Squid.”
Monday, August 24	Dr. R. Weissenberg	“The Lymphocystic Disease of Fishes and Its Significance for Intracellular Parasitism: a Contribution to the Knowledge of the Virus Diseases.”
Thursday, September 3 (Under the joint auspices of the Genetics Society of America and the Marine Biological Laboratory)	Dr. Th. Dobzhansky	“Genetic Nature of Species Differences.”
	Dr. Lee R. Dice	“Some Types of Waltzing and Epilepsy in Mice of the Genus Peromyscus.” (Motion Picture.)
Friday, September 4	Dr. Johan Hjort	“Distribution of Marine Animals in Relation to Their Environment.”

6. SHORTER SCIENTIFIC PAPERS, 1936

Tuesday, June 30	Dr. Matilda M. Brooks	“The Effect of Methylene Blue on the Spectrophotometric Picture of Hemoglobin, CO-Hemoglobin and CN-Hemoglobin.”
	Mr. Harry J. Lipman	“The Phosphatase Content of the Developing Chick Embryo.”

Dr. Kenneth C. Fisher and
Dr. Laurence Irving .... "The Description of an Oxidative Process Maintaining the Frequency of the Heart Beat."

Dr. Eric G. Ball .... "Oxidation-reduction Potentials and Potentiometric Determination of Ascorbic Acid."

Tuesday, July 7

Dr. Alfred M. Lucas .... "Nerve Cells Without Central Processes in the Fourth Spinal Ganglion of the Frog."

Dr. José F. Nonidez .... "Receptor Areas in the Venae Cavae and the Pulmonary Veins and Their Relation to Bainbridge's Reflex."

Dr. C. Ladd Prosser .... "Extinction of Reflex Responses in the Rat."

Dr. R. W. Gerard .... "Factors Influencing the Electrical Activity of the Brain."

Tuesday, July 14

Dr. A. K. Parpart .... "The Permeability of the Erythrocytes of the Ground-hog."

Dr. H. Burr Steinbach .... "Effect of Salts on the Injury Potentials of Frog's Muscle."

Mr. Daniel Mazia and
Dr. Jean M. Clark .... "Free Calcium in the Action of Stimulating Agents on Elodea Cells."

Dr. Kurt G. Stern and
Dr. Delafield Dubois .... "A Photoelectric Method for Recording Fast Chemical Reactions: Application to the Study of Catalyst-Substrate Compounds."

Tuesday, July 21

Dr. W. W. Ballard .... "Observations on Lens Regeneration in Amblystoma."

Dr. T. H. Bissonnette .... "Fertile Eggs from Pheasants in January by Night-lighting."

Dr. Roberts Rugh .... "A Quantitative Analysis of the Anterior Pituitary-Ovulation Relation in the Frog."

Tuesday, July 28

Dr. Gertrude Evans .... "The Relation between Vitamins and the Growth and Survival of Goldfishes in Homotypically Conditioned Water."

Dr. C. P. Kraatz .... "A Possible Endocrine Rôle of the Eosinophil Leucocytes in the Female Rat."

Dr. J. E. Kindred .... "An Interpretation of the Secondary Lymphoid Nodules in the Albino Rat."

Dr. Laurence Irving	"Physiological Adjustments to Diving in the Beaver."
Tuesday, August 4	
Dr. Ethel Browne Harvey	"Development of Arbacia Eggs without Nuclei: Parthenogenetic Merogony."
Mr. F. Moser	"Cortical Changes in Arbacia Eggs During Fertilization—A Moving Picture."
Dr. H. J. Fry	"Temperature Effects on Mitotic Changes in Arbacia Eggs."
Dr. Sven Hörstadius	"Sea-urchin Larvæ with Cytoplasm of One Species and Nucleus of Another."
Tuesday, August 11	
Dr. Laura J. Nahm	"A Study of the Cells of the Adrenal Gland of the Ewe during Estrus and Pregnancy."
Dr. E. Alfred Wolf and Miss Grace Riethmiller	"Studies in Calcification: III. The Shell of the Hen's Egg."
Dr. Alexander Sandow	"Diffraction Patterns of Striated Muscle and Sarcomere Behavior during Contraction."
Dr. Frank H. J. Figge	"The Effect of Some Oxidation-reduction Indicator Dyes (Phenol Indophenol) on the Eyes and Pigmentation of Normal and Hypophysectomized Amphibians."
Dr. Robert Chambers	"The Elimination of Neutral Red by the Kidney Tubules."
Tuesday, August 18	
Dr. H. P. Smith and Dr. E. D. Warner	"Quantitative Studies on Blood Clotting."
Dr. Alan C. Burton	"The Basis of the Principle of the Master Reaction in Biology."
Dr. C. S. French	"Efficiency of Photosynthesis in Purple Bacteria."
Dr. Alexander Hollaender	"Some Effects of Ultraviolet Radiation on Bacteria."
Tuesday, August 25	
Dr. E. Eleanor Carothers	"Cellular Behavior in Abnormal Growths Produced by Irradiation of Grasshopper Embryos."
Dr. Katsuma Dan, Mr. T. Yanagita and Mr. M. Sugiyama	"The Behavior of the Cell Surface During Cleavage."
Dr. T. H. Bissonnette	"Modified Sexual Photoperiodicity in Ferrets, Raccoons and Quail."

Dr. Earl A. Martin	"Asexual Reproduction in Dodecaceria fimbriatus."
Mr. G. P. Wells	"The Physiology of the Stomatogastric System in Arenicola marina."

7. GENERAL SCIENTIFIC MEETING, 1936

Thursday, August 27

Mr. F. R. Hunter and Dr. E. N. Harvey	"The Effect of Lack of Oxygen on the Permeability of the Egg of Arbacia punctulata."
Dr. B. Lucké, Mr. R. Ricca and Dr. H. K. Hartline	"Comparative Permeability to Water and Certain Solutes of the Egg Cells of Three Marine Invertebrates (Arbacia, Cumingia and Chaetopterus)."
Mr. S. A. Corson	"Permeability of Ameba proteus to Ions."
Dr. F. J. M. Sichel and Dr. A. C. Burton	"A Kinetic Method of Studying Surface Forces in the Egg of Arbacia."
Dr. R. Chambers	"Experimental Studies on the Oil-wetting Property of the Plasma Membrane."
Dr. M. J. Kopac	"Interfacial Films between Oil and Cytoplasm."
Dr. P. S. Henshaw	"The Question of Recovery from X-ray Effects in Arbacia Sperm."
Miss Anna K. Keltch, Dr. G. H. A. Clowes and Dr. M. E. Krahl	"The Respiratory Effects Exerted by Certain Organic Compounds in Relation to Their Molecular Structure."
Dr. M. E. Krahl, Dr. G. H. A. Clowes and Mr. J. F. Taylor	"Action of Metabolic Stimulants and Depressants on Cell Division at Varying Carbon Dioxide Tensions."
Dr. W. C. Allee and Dr. Gertrude Evans	"Further Studies on the Effect of Numbers Present on the Rate of Cleavage in Arbacia."
Dr. A. K. Parpart and Dr. M. H. Jacobs	"Paradoxical Osmotic Volume Changes in Erythrocytes."

Dr. M. H. Jacobs, Mr. H. N. Glassman and Dr. A. K. Parpart	"Further Studies on Specific Physiological Properties of Erythrocytes."
Friday, August 28	
Dr. C. C. Speidel	"Experiments on the Contractile Substance of Muscle Fibers." (With Motion Pictures.)
Miss Elsa M. Keil and Dr. F. J. M. Sichel	"The Injection of Aqueous Solutions, Including Acetylcholine, into the Isolated Muscle Fiber."
Dr. E. Bozler	"The Double Refraction of Smooth Muscle."
Dr. F. O. Schmitt, Dr. R. S. Bear and Mr. J. Z. Young	"Some Physical and Chemical Properties of the Axis Cylinder of the Giant Axons of the Squid, Loligo pealii."
Mr. G. Schoepfle and Mr. J. Z. Young	"The Structure of the Eye of Pecten."
Dr. H. K. Hartline	"The Discharge of Impulses in the Optic Nerve Fibers of the Eye of Pecten irradians."
Dr. K. C. Fisher and Dr. J. A. Cameron	"The Effect of Light on the CO-poisoned Embryonic Fundulus Heart."
Dr. G. Saslow	"Prevention of Edema in Frog Perfusions in the Absence of Serum Proteins."
Dr. J. A. Cameron and Miss K. O. Mills	"Behavior of Frog Tadpole Epidermal Cells during Seven Successive 24-Hour Regeneration Periods."
Dr. E. R. Clark and Mrs. Eleanor L. Clark	"Observations on Conditions Affecting Growth of Cells and Tissues, from Microscopic Studies on the Living Animal."
Miss Laura N. Hunter	"Some Nuclear Phenomena in the Trichodina (Protozoa, Ciliata, Peritrichida) from Thyone briareus (Holothuroidea)."
Dr. S. Hörstadius	"Investigations on Determination in the Early Development of Cerebratulus."

Dr. R. Rugh .... "Preliminary Evidence as to a Source of the Growth and the Sex-stimulating Hormones in the Bullfrog."

Dr. P. B. Armstrong .... "Mechanism of Hatching in Fundulus heteroclitus."

Dr. B. H. Grave and
Mr. J. Smith .... "Hermaphroditism and Sexual Inversion in Mollusca."

PAPERS READ BY TITLE

Dr. H. G. duBuy .... "Separation of the Conducting and Contractile Elements in the Retractor Muscle of Thyone briareus."

Dr. A. V. Hunninen and
Dr. R. Wichterman .... "Hyperparasitism: A Species of Hexamita (Protozoa, Mastigophora) Found in the Reproductive Systems of Deropristis inflata (Trematoda) of Marine Eels."

Dr. Mary S. MacDougall ...... "Cytological Studies of the Genus Chilodonella. III. The Conjugation of Chilodonella labiata, variation?"

Dr. Victor Schechter .... "Comparative Hypotonic Cytolysis of Several Types of Invertebrate Egg Cells and the Influence of Age."

Dr. H. W. Stunkard .... "Notes on Life Cycles of Digenetic Trematodes."

Dr. A. J. Waterman .... "Inhibition of Gastrulation in Arbacia with $NiCl_2$."

Dr. R. Wichterman .... "Division and Conjugation in Nyctotherus cordiformis (Ehr.) Stein (Protozoa, Ciliata) with Special Reference to the Nuclear Phenomena."

DEMONSTRATIONS

Thursday, August 27

Dr. F. H. J. Figge .... "The Effect of Some Indophenol Dyes on the Eyes and Pigmentation of Various Amphibian Larvæ."

Dr. R. Weissenberg .... "Intracellular Parasitism of Microsporidia and of Lymphocystic Disease."

Dr. S. E. Pond	“A Semi-portable Cold Room Providing Daylight and Automatic Adjustment of Temperature from — 10° C. to Room Temperature.”
Miss Laura N. Hunter	“Some Nuclear Phenomena in the Trichodina from Thyone briareus (Vegetative Stages, Binary Fission, Conjugation (?)).”
Dr. A. Hollaender	“A Simple Intense Mercury Vapor Lamp.”
Dr. Ethel B. Harvey	“Cleavage of Non-nucleate Arbacia Eggs.”
Dr. E. R. Clark and Mrs. Eleanor L. Clark	“Observations on Conditions Affecting Growth of Cells and Tissues, from Microscopic Studies on the Living Animal.”
Miss Gertrude Gottschall	“The Determination of Glutathione in Animal Tissues.”
Dr. D. Marsland	“A Bomb Permitting the Microscopic Observation of Cells and Tissues during Hydrostatic Compression. The Effects of Compression on the Cleavage of Arbacia Eggs.”
Dr. S. E. Hill	“Action Currents in Nitella.”
Mr. F. R. Hunter	“Apparatus for Determining Volume Changes of Cells under Anærobic Conditions.”
Dr. H. W. Stunkard	“Life Cycles of Digenetic Trematodes.”
Mr. J. Z. Young	“Anatomy and Physiology of Giant Nerve Fibers in the Squid.”
Dr. E. Bozler	“Method Recording Changes of Double Refraction during Muscular Contraction.”
	“Sensitive Method of Recording Responses of Blood Vessels.”
Dr. H. K. Hartline	“The Discharge of Impulses in the Optic Nerve Fibers of the Eye of Pecten irradians.”
Dr. M. N. Knisely	“Microscopic Demonstration of Several Living Internal Organs of Frogs Illuminated with Fused Quartz Rods.”

8. MEMBERS OF THE CORPORATION

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PLACOID SCALE TYPES AND THEIR DISTRIBUTION IN SQUALUS ACANTHIAS

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Detailed studies of the development of placoid scales were reported by Hertwig (1874). His descriptions of certain integumentary scales and of stomodeal denticles have been supplemented by Steinhard (1903), Imms (1905) and Radcliffe (1916). These workers give no indication that *Squalus* has more than a couple of different types of integumentary scales or that scaleless regions occur. Most text-book statements imply that dogfish scales all have pointed spines. For instance, Wilder (1923, p. 82) says: " The scales in the dogfish are of the form known as placoid, each consisting of an approximately flat base from which rises a sharp-pointed cusp, inclined in the direction of the free edge of the scale, or posteriorly when the scale is in place." Sakamoto (1930), however, has reported several different shapes of scales in five Japanese species of sharks.

The work reported here was undertaken after it had been noted that there was considerable variation in the scales of pieces of dogfish skin taken at random for demonstration to students. An attempt is here made to describe the types, distribution and orientation of scales of specimens of *Squalus acanthias* of 54 to 62 cm. length.

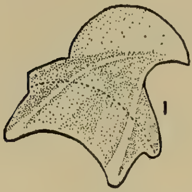
For this work, the skin was removed in relatively large pieces, the exact orientations and positions of which were noted. In all cases the same regions of both sides of the body were compared. Detailed studies of 7 specimens were made. Several other fish were used as additional checks of certain points. At first these were studied after clearing in methyl salicylate without staining. Later several were prepared by staining in alizarin red S and then clearing. In addition small portions of the skin, representative of different scale-types, were macerated in 0.5 M NaOH at a temperature of about 85° C. Isolated scales were thus obtained.

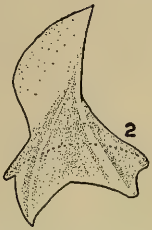
OBSERVATIONS

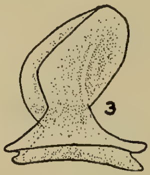
For purposes of convenience the integument may be considered as belonging to the following regions, each of which will be considered separately: trunk and tail regions of the body proper, as contrasted with head; fins, including spine pockets and, in male, claspers; head; mouth opening and labial pouches; ampullæ of Lorenzini; olfactory sacs; eyelids; spiracles; external gill slits; internal gill slits; mouth cavity and pharynx.

The placoid scale of *Squalus acanthias* consists of a basal plate and spine (as noted by Hertwig and many others). The basal plate (except in the case of stomodeals and combs) is quadrangular with two angles extending laterally, one anteriorly and a fourth posteriorly. The stomodeal basal plate also usually bears four extensions, but the lateral ones, as well as the anterior, extend anteriorly. The basal plate of the comb type is similar to that of stomodeals but the angles are often indefinite and the whole basal plate frequently lacks the regularity usually found in this part of other scales.

The spine may be interpreted as consisting of two main elements: (1) a longitudinal plate which extends, in plan, between anterior and posterior angles of the basal plate; and (2) a transverse plate which extends between the lateral angles of the basal plate. The transverse component is ordinarily tilted posteriorly and rests on the posterior part of the longitudinal component. The anterior part of the latter extends onto the transverse element at least to some extent. Scale types are associated with more or less marked variations in the development and shapes of these spine components.







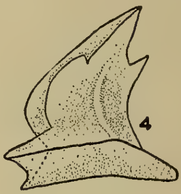


FIG. 1. Left lateral aspect of a dorsal body type scale. × 75.
FIG. 2. Right lateral aspect of a ventral body type scale. × 75.
FIG. 3. Postero-lateral aspect of a transitional type scale. × 75.
FIG. 4. Postero-lateral aspect of a body tricuspid scale. × 75.

It is well known that an opening on the under side of the basal plate connects with a system of dentinal canals which extend even into the spine. The openings and canal systems are not shown in the drawings.

Trunk and Tail Regions of Body Proper

On the trunk and tail regions of the body proper there are four general types of scales: (1) dorsal body type (helmet-scale of Hertwig);

(2) ventral body type; (3) transitional type; (4) tricuspid type, showing various degrees of development of the lateral cusps. These divisions are not sharp, many intermediate forms occurring.

In the dorsal body type (Fig. 1) the transverse component is more than half as wide as the basal plate; the part of the longitudinal element anterior to the transverse element is of about the same width as the latter; the posterior part of the longitudinal element is small; and the entire spine is about as high as it is wide. In the ventral body type (Figs. 2 and 14) the transverse component and the anterior part of the longitudinal component are about two-thirds as wide as in the dorsal type; the height of the spine, on the other hand, is about fifty per cent greater than in the dorsal type. The so-called transitional type (Fig. 3) is intermediate between dorsal and ventral types. The tricuspid scales (Figs. 4 and 18) are of about the same proportions as the dorsal and transitional types but bear two lateral, secondary cusps set some distance anterior to the tip of the scale and varying greatly in size in different scales.

Along the mid-dorsal line there are several (3–5) rows of large, heavy scales in which tricuspids predominate but occasional dorsal body scales are present. The dorsal and dorso-lateral surfaces bear dorsal body type and tricuspids. The latter vary from scales with large secondary cusps to some with these so small that the scales may be interpreted as intermediate between tricuspids and dorsal body type. From the level of the lateral line through the region of reduction in integumentary pigmentation, the dorsal body type grades over, through the transitional type, to the ventral body type (Fig. 16). In this transition zone the tricuspids become more and more scarce until, in the practically white ventral part of the body, nearly all scales are of the ventral body type. In this transition zone the basal plates are about twenty per cent smaller, both in length and in width, than they are either dorsally or ventrally. This smaller size, coupled with a slightly greater scattering of scales in the transition zone, gives the impression that there is a relatively great reduction in the number of scales here.

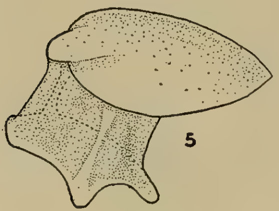
Neither the type nor the arrangement of scales is particularly altered along the lateral line (Fig. 16). Also no significant size-differences exist between anterior and posterior levels.

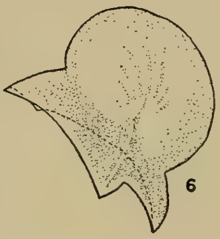
Body scales all point posteriorly. Frequently several may be somewhat deflected so that the long axis is slightly oblique. The general pattern of distribution shows the scales to be arranged in diagonal rows, the scales of each row lying between and behind two of the preceding row (as noted by Klaatsch, 1890, and many others). Under high magnification, however, it is at once evident that the scales are not arranged in a precise geometric fashion. This is due to the fact that the rows are not exactly parallel, the scales vary in size, and " extra " scales are quite numerous, thus disarranging the pattern.

Fins

The *pectoral fin* will be described in detail first. The other appendages will then be described only in so far as they differ from the pectorals.

The pectoral fin has a heavy anterior margin, a thinner axillary one and a curved, filamentous distal one. The anterior and axillary extreme margins, where the skin of the dorsal side of the fin passes over into that of the ventral, both bear scales. The anterior border is covered by closely arranged and frequently overlapping fin-marginal scales (Figs. 5 and 19). Each of these scales is relatively large with a large, high pedicel. The massive, transverse spine-component is tilted so far that its surface is nearly parallel with the surface of the integument. The posterior tip of this is only slightly pointed. A short, thick part of the anterior longitudinal component projects from the anterior end of the transverse element. Toward the distal end of the fin the spines of these scales become smaller and somewhat less massive. Extending from the entire length of this anterior border toward the middle of the fin there is a zone of transition to the common fin type which is similar to the dorsal body type but with the average scale smaller. Following through this zone from the anterior border, we find first the fin-marginals more disperse than on the border, then transitionals and body tricuspids appearing, and finally the common fin type. This transition zone extends onto the body of the fish a very short distance.





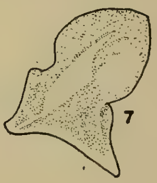


FIG. 5. Lateral aspect of a fin-marginal scale. × 75.

FIG. 6. Marginal type scale from vicinity of axillary scaleless area. × 75.

FIG. 7. Paramarginal scale from region slightly farther from axillary scaleless area than that occupied by scale in Fig. 6. × 75.

The arrangement of scales on the axillary border is complicated somewhat by the presence of a scaleless area at the base of the fin here.

The extent of this area is less on the fin than on the body wall. It is shaped somewhat like a low, thick *J* with the long part extending along the dorsal side of the fin-body junction, the loop around the posterior side and the short part on the ventral side for a short distance. This scaleless area is surrounded by several rows of marginal scales (Figs. 6 and 17). These scales have a large, rounded transverse component with the longitudinal component reduced posteriorly and almost lacking anteriorly. Next to these scales are rows of paramarginals (Fig. 7) with smaller and less rounded transverse components than have the marginals. Both of these types are more numerous on the body than on the fin. They are also less numerous at the anterior ends of the scaleless area where they may even be lacking. At the anterior ends of the ventral and dorsal parts of this area the spines point considerably away from it. In the posterior part they return gradually to their ordinary orientation. This arrangement is, however, subject to some variation. Several spines may point toward the scaleless area, for example. The basal part of the axillary border of the fin has paramarginal scales for a short distance, always greater on the dorsal side than on the ventral, probably associated with the difference in extent of the scaleless areas on the two sides. The remainder of the axillary border is covered with scales similar to those on the anterior but slightly smaller. The zone of transition toward the common fin type is narrower here than at the anterior border.

The general transition from body to fin is not associated with a change in scale type. The dorsal body type scales, found here, are smaller than on the body wall. This is especially true near the distal margin of the fin. Most of the fin is covered by this common type, more closely set than on the body wall and arranged in a more regular pattern. The scales extend to the very edge of the filamentous border which is covered by small transitional and common fin types and, at the very edge, tricuspids. These tricuspids are slightly larger than other scales here.

The scales of the anterior and axillary borders curve to follow these borders but not exactly so. The result is that the spines at the very edges extend off the margins. Except at the borders, the orientation on the fin does not follow the curvature of the fin rays but is in straight lines toward the filamentous border.

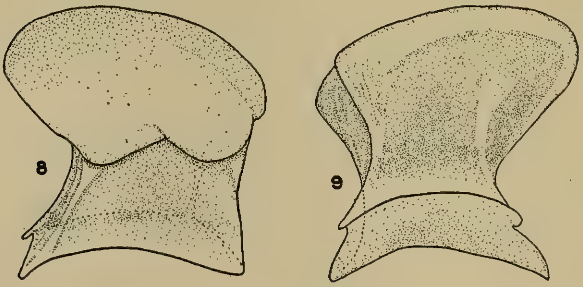
On the *anterior* and *posterior dorsal fins* the transition zone at the anterior margin extends onto the body anterior to the spine pocket which is covered with massive, shingling fin-marginals. The anterior margin of the fin is devoid of scales immediately behind the basal portion of the spine (Fig. 19). This small scaleless area is surrounded by marginal or paramarginal scales. There is also a scaleless area at the posterior attachment of the fin. This area is small, symmetrical, with rounded margins, and surrounded by marginal and then paramarginal scales.

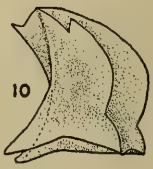
On the *pelvic fins* the fin-marginal scales are less massive than on other fins and the median borders are almost entirely covered by paramarginal scales. The cloaca is scale-free. There is a large scaleless area extending around the cloacal opening and continuing along the attachments of the median borders of the fins and onto their dorsal surfaces which are in contact with the body wall. This area does not extend onto the ventral surfaces of the fins. Around this scaleless area are marginal and then paramarginal scales. In the male the ventral side of each clasper bears marginal scales at its posterior end. The anterior end bears marginals medially, tricuspids laterally and paramarginals in transition between them on the ventral side. These scales all point medially and are closely arranged. The dorsal side of each clasper bears no scales, this scaleless area being continuous with that around the cloaca.

The *caudal fin* has no scaleless area associated with it. Transitional and tricuspid scales are more numerous than on other fins, especially toward the dorsal side.

Head

The snout is covered by a characteristic snout type scale (Figs. 8, 9 and 15). Each of these scales, like fin-marginals, is relatively large with a large, high pedicel and with a massive transverse spine-component tilted so that its surface is nearly parallel with the surface of the integument. The entire margin of this component is robust and the posterior end is rounded. In many cases there is a distinct overlapping of these scales. All snout scales radiate from a point at the extreme anterior tip of the snout but slightly dorsal. Laterally this type extends farther to





FIGS. 8 and 9. Different aspects of two snout type scales. × 75.
FIG. 10. Postero-lateral aspect of a head tricuspid scale. × 75.

the posterior and is also more numerous than on either dorsal or ventral surfaces. These scales extend posteriorly for several millimeters where they merge into the head type (Figs. 10 and 20). Scales of the latter type are thick-set and have relatively short tricuspid spines with secondary cusps nearly as large as the main one. They become somewhat smaller and less numerous posteriorly. Near the dorsal midline they are oriented with the spine pointing directly toward the posterior. On either side of this region the spines have a slightly lateral orientation. At the level of the spiracle the head type is mixed with the dorsal body type. The spiracle opening, however, is usually surrounded by the head type for the most part. At the level of the first functional gill slit the transition to body type is usually complete. On the ventral side there are, in addition to the head type, scales similar to the snout type but less massive. The region of transition to ventral body type, on the ventral side, is relatively broad and is complicated by the presence of the mouth opening. The transition is completed a short distance posterior to the lower jaw.

Mouth Opening and Labial Pockets

There is usually a very narrow scaleless area just outside the rows of teeth. This is frequently absent near the mid-line but widens out toward the angles of the jaws. In the upper jaw region the integument adjacent to this scaleless area possesses one or two rows of marginal scales anterior to which are several rows of the paramarginals. These scales have interrupted those of the ordinary head type which are prevalent anterior to them. On the lower lip is a row, or two, of scales quite similar to the snout type, posterior to which scales of the head type occur again. Scale orientation is not affected by the presence of the lips.

The walls of the labial pouches are scaleless. No marginal or snout-type scales bound this area. Instead, the same type of scale is found here as occurs in this general region of the head, namely: paramarginals bounding the anterior part of the pouch adjacent to the upper jaw and head type along the posterior part. Apparently the only modification in scales in the vicinity of these pouches is in their orientation which is changed so that the spines follow the margins of the pouches and point postero-laterally.

Ampullæ of Lorenzini

The presence of the numerous openings of the ampullæ of Lorenzini on the head does not bring about any distinct local changes in scale-type (Fig. 20). The spines are often shaped to curve about the margins of the openings when parts of the basal plates would ordinarily be in the area which the openings occupy. This condition is more common dorsally than ventrally. Often a spine overlaps an opening.

Olfactory Sacs

There is usually no modification of scale type about the margin of an olfactory opening. Head type scales are found here. The scales do not end abruptly but continue into the cavity a short distance. Posterior orientation is maintained mediad but not laterad to the opening. Some of the scales antero-lateral to the opening are turned medially toward the anterior flap, others laterally to follow the lateral margin of the aperture. The anterior flap is covered by scales similar to the head type but considerably smaller and more deeply serrate. They are very closely arranged and point posteriorly. The smaller posterior flap is covered by similar scales, somewhat larger, closely arranged and pointing posteriorly.

Eyelids

The dorsal and ventral eyelids do not differ as far as the scales are concerned. The scales covering the margin of an eyelid are of several types. Part of the lid is covered by head type and part by a mixture of small transitional and tricuspid scales. These scales are smaller than those of the surrounding area and much more closely arranged, being almost shingled. The scales curve about the margins, especially anteriorly. The scales are confined to the external margins, none being found on the edge of the conjunctiva.

Spiracles

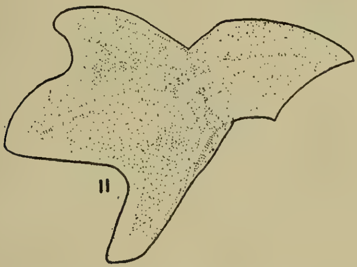
The external opening of the spiracle is entirely surrounded by head type scales, smaller and more closely grouped than elsewhere in that vicinity. At the anterior side the scales are deflected away from the middle of the opening. In those cases where the head-body transition extends to this level the spiracle may be surrounded by the several types. No integumentary scales enter the opening.

The posterior wall of the spiracular cavity bears numerous typical stomodeal denticles (Fig. 11) somewhat like those described by Hertwig, Steinhard, and others. Following anteriorly around the wall, along the dorso-medial side, the denticles become less numerous up to the mid-dorsal part, then more abundant until they are usually almost as abundant in the anterior wall as in the posterior. The ventro-lateral wall is without denticles. There is no particular transition into this area from either anterior or posterior sides. Instead, the denticles stop rather abruptly. Also the denticles are less numerous near the external opening which they approach very closely. The points of these scales are directed toward the pharynx.

External Gill Slits

The scales covering the distal, exposed ends of the gill septa are of several types, dependent on the location of the septum. The scales covering the whole anterior surface of the first flap—in front of the first functional gill slit—are of the type on the body wall nearby but smaller. They are usually of dorsal body type, but sometimes transitionals. The posterior surface of the septum is scaleless nearly to the gill filaments. A narrow area adjacent to the filaments has comb type scales (Figs. 12 and 13) such as have already been described by Steinhard (1903). These are very few in number and haphazardly arranged.

The distal, exposed ends of the septa of the four holobranchs are alike. The anterior surface, at its free border, is covered usually by dorsal body type scales, often interspersed with transitional and tricuspid types, sometimes with small head type or paramarginals. There is a transition through paramarginals to marginals internally up to the point where the septum is covered by that in front. Then there is a scaleless region. Beyond the latter there are comb scales similar to those on the posterior face of the first flap. The posterior faces of these septa are similar to that of the first. There are wider areas bearing comb scales on the anterior than on the posterior faces, possibly associated with the fact that anterior demibranchs do not extend externally as far as do posterior ones. The extra part of the smooth septum in each case bears comb scales. No scales occur on the gill filaments.



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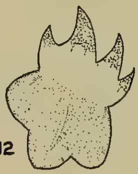

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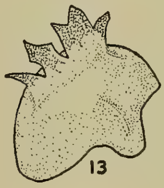

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FIG. 11. Stomodeal denticle from floor of pharynx. × 75.
FIGS. 12 and 13. Two comb scales from wall of a gill slit. × 75.

The posterior wall of the fifth gill pocket bears no filaments and no comb scales near the surface of the body. A few, scattered, marginal scales are found in the outer part of the area covered by the flap of the last holobranch. Where exposed, really the general surface of the body at that point, there are at first marginal scales. The latter merge posteriorly into typical fin-marginal scales, probably associated with the adjoining pectoral fin.

Internal Gill Slits

The scales on the membrane which covers each gill arch are of two distinct types (as reported by Steinhard, 1903). One, a typical stomodeal denticle, is found toward the pharyngeal side of the arch and on

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PLATE I

Surface view photomicrographs of pieces of integument of a *Squalus acanthias* 55 cm. long. In all cases the top of the figure is the anterior end and the spines point toward the bottom of the page. Figure 17 is about × 50, all others about × 40.

FIG. 14. From the ventral, mid-trunk region. The basal plates show as gray backgrounds for the spines. Compare Fig. 2.

FIG. 15. From dorsal side of snout near point from which all scales radiate. The "heart-shaped" surface of one scale—in the lower right quarter—has been outlined. The limits of another—near the center—have also been indicated. Overlapping obscures to some extent the limits of many scales. Slightly to the left and below the center of the figure there is a scale with the anterior parts of the pedicel and basal plate clearly outlined as they recede from the notched anterior margin of the scale surface. Compare Figs. 8 and 9.

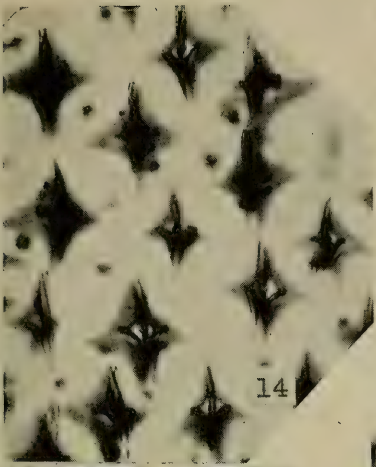
FIG. 16. From the lateral line area in the mid-trunk region. Two openings of the lateral line canal show in the center.

FIG. 17. From the side of the trunk in the axillary region. At the right side of the top of this figure the very edge of the axillary scaleless region shows. A scale in the lower right quarter has been outlined in stipple. The solid black areas are the basal plates. Compare Fig. 6.

FIG. 18. From the dorso-lateral part of the mid-trunk region. The two scales in the upper corners are slightly modified dorsal body type. All others are tricuspids. To the right of the center is one which has been outlined in stipple. Compare Fig. 4.

FIG. 19. From the region immediately distal to the scaleless area behind the base of the spine of the antero-dorsal fin. The very tip of this scaleless area shows at the center of the top edge of the figure. Two scaleless patches also show, one in the center and the other near the bottom. This strip shows the transition from the ordinary marginal type of scale—at the top—to the heavy fin-marginal type. In the lower left corner is one of the latter which shows clearly the ramifications of the portions of the pulp cavity which extend into the "heart-shaped" outer part of the spine. Compare Fig. 5.

FIG. 20. From the dorsal side of the head just anterior to the eyes. The opening of an ampulla is shown at the top. Note that the spines of scales around the opening are still oriented posteriorly. In the lower right corner a scale has been outlined. To the left of the latter the general form of a head tricuspid shows clearly. Compare Fig. 10 and also body tricuspids in Fig. 18.



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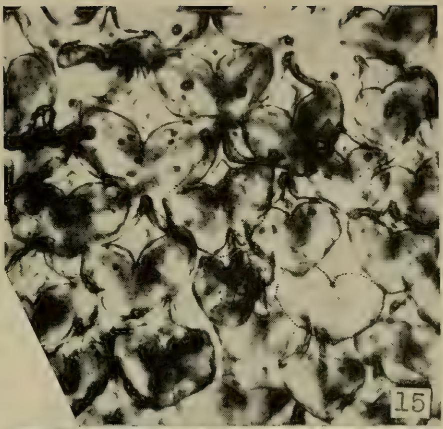

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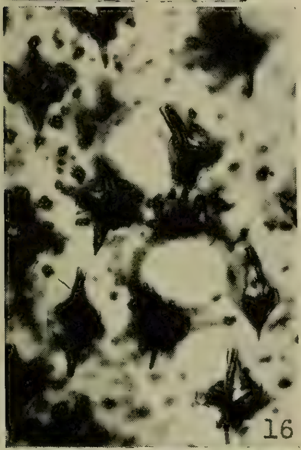

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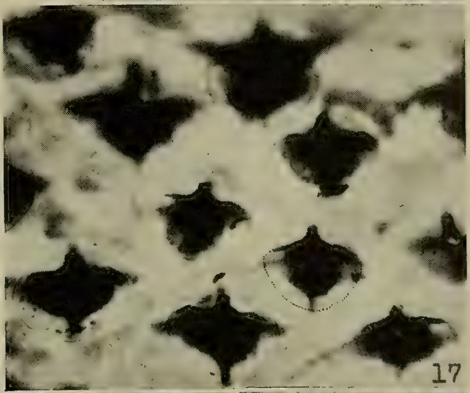

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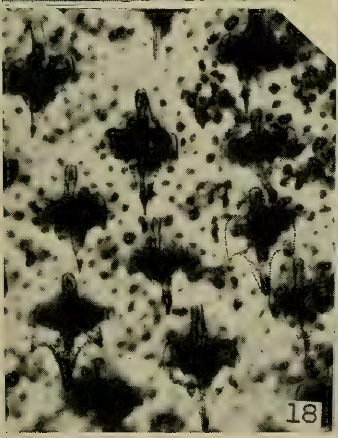

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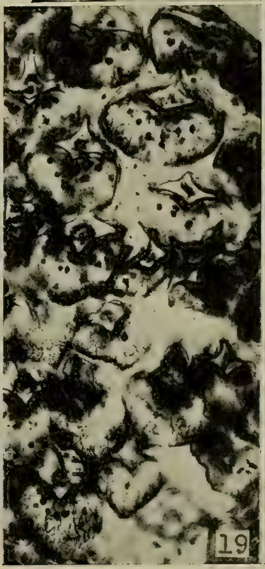

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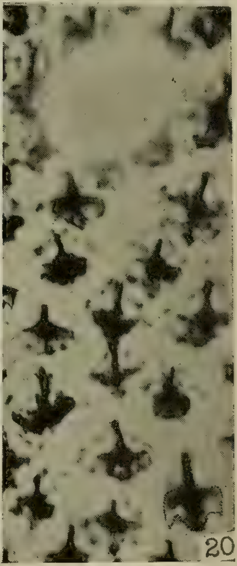

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Plate I

the gill rakers. These scales are never very numerous, but are more abundant on the anterior than on the posterior side. Usually there are more toward the dorsal than toward the ventral end of the arch and more on the rakers than on the adjacent portions of the arch. The spines point toward the pharynx. There is no distinct arrangement plan of the scales.

The second type is the comb scale, similar to that found just external to the filaments. These scales are smaller than the stomodeals and very much more numerous. They occur on the side of the arch toward the filaments, being almost exclusively confined to the anterior surface along its whole length. When present on the posterior surface, they are confined to two small areas—one dorsal and one ventral—where the membrane of the anterior side of one arch passes over into that of the posterior side of the arch in front.

This situation holds true for all four holobranchs. The anterior wall of the first functional gill slit bears only combs at the extreme dorsal and ventral ends, as mentioned for the other slits. The posterior wall of the fifth slit bears no filaments but does have many comb scales, not only in the inner part but also scattered over much of the area which corresponds to that occupied by filaments in other slits.

As with the stomodeal type, the combs all point toward the pharynx. They are usually several rows wide (4–6), frequently with each scale lying behind and between two others. This arrangement is not precise as the rows are somewhat irregular, the sizes of the scales vary and " extras " occur.

Mouth Cavity and Pharynx

There are no scales on the roof of the mouth or pharynx. The few scales on the dorso-lateral part of the pharynx are associated with the membrane covering the gill arches rather than that of the pharyngeal roof. The floor of the pharynx bears typical stomodeal denticles in a restricted area which begins in the mid-ventral line at the level of the first functional gill slit, or slightly anterior to it, and extends as far posterior as the last slit. This area is usually quite narrow near its anterior end but is about half the width of the floor of the pharynx throughout most of its length. The lateral quarter of the floor, on each side of this scale-bearing region, is ordinarily scaleless. In some specimens a few scales may be present in the posterior parts of these lateral areas. These stomodeal denticles are much sparser than body scales and are irregularly oriented, although the majority point posteriorly. They are haphazardly arranged.

Discussion

Most descriptions of the integumentary scales of *Squalus acanthias* have been confined to two types: (1) the general body type, mainly of the dorsal variety; and (2) the tricuspid, also called tridentate. Radcliffe (1916) described but one type in the integument of this animal, namely, the tridentate. In his Fig. 20 he showed twenty-one scales, all obviously of this type. This is the type which would ordinarily be present in a piece " from the middle of the side below the first dorsal fin," which Radcliffe—in a later paper (1917)—suggested using for identification purposes. These scales resemble closely those of *Scyllium* described by Klaatsch (1890) whose sketches have been used frequently as a source in depicting placoid scales. The general body type of *Squalus* was early described by Hertwig (1874) and later by Steinhard (1903). We have observed scales of both of these types in considerable numbers on the trunk and tail regions of this animal. The body type really exists in two forms, both of which have similar basal plates. The ventral body type, however, differs from the dorsal in having a considerably higher spine, both components of which are much narrower than in the dorsal type.

In addition to these two types, we have observed at least two other main types. One of these is a large scale with large pedicel and massive transverse spine-component the surface of which lies nearly parallel with the body surface. The other is a marginal type consisting of a stellate base, an abbreviated pedicel and a spine made up, for the most part, of a large, circular transverse-component, the longitudinal elements being much reduced. In addition there are scales of several other shapes which should perhaps be regarded as intermediates between other types since they are found in places which may be considered transition zones.

The massive scales are apparently of two distinct varieties. One is the so-called snout type found on the tip of the snout and on the tip of the lower jaw. The other, the fin-marginal, is on the anterior margins of all fins except the parts of the antero- and postero-dorsals which are protected by the fin-spines. Both of these varieties tend to be set close together and to overlap in many cases. It would appear that this is a special protective type found especially on anteriorly exposed surfaces.

The ordinary marginal scales surround scaleless areas where one part of the actual surface of the body covers another. Such areas include: those in the two axillary regions (on the body wall and on the base of each pectoral fin along the posterior portion of the attachment of the latter); those beneath the posterior portions of the two dorsal fins; those behind the spines of these dorsal fins; those beneath the external flaps of the gill septa; and a very large area along the dorsal sides of the pelvic fins, on adjacent parts of the body wall and around the cloaca. No mention of these scaleless areas has been made by other workers, including Sakamoto (1930), who made a thorough study of several species of Japanese sharks. On the other hand, those scaleless areas which are inside of various invaginations, in most pores and in other openings are usually not surrounded by special marginal scales but instead by the type commonly found in the region in question. Included among these places are the ampullæ of Lorenzini (Fig. 20), the pores of the lateral line canals (Fig. 16) and associated canals of the acustico-lateral system of the surface of the head, the labial pockets, the spiracles, the olfactory sacs, and the inpocketings to form the eyelids.

Sakamoto (1930) has depicted a number of types of scales some of which resemble certain of these of *Squalus*. In describing the scales of *Cynias manazo,* he reported that the " ridges of the scales on the dorsal side of the trunk are more massive and higher than those of the scales on the ventral side of the same." In *Squalus* there exists a similar condition of the single ridge which we have called the anterior part of the longitudinal spine-component. None of the scales of *Squalus* seem to bear more than a single such ridge. Rudiments of this element are present on most scales of *Squalus*. They were confined to the trunk and caudal scales of four of the five species studied by Sakamoto. In *Carcharinus japonicus,* ridges were also present on scales of the buccal cavity. This worker also reported that in general in *Cynias* " the width of the basal plate (is) proportional to that of the whole placoid scale." In *Squalus* such is not the case. For example, the spines of ventral trunk scales are much more narrow than those of the dorsal type. The basal plates, however, are of approximately the same size and shape in both types. Also, in the lateral transition zone, where the spines are intermediate in size, the basal plates are considerably smaller than those of either dorsal or ventral scales.

Sakamoto also found that in *Cynias* " the dimension of the scale is the largest in the trunk, larger on the head, and the smallest on the tip of the fins." In *Squalus,* on the other hand, scales of the snout type are the largest and fin-marginals are next in size. Those on the tips of the fins are smallest, as in *Cynias*.

Steinhard's observations on the structure of the comb scales of the gill cavities have been verified by us. On the other hand, the stomodeal denticles described and depicted by Hertwig and by Steinhard do not seem to match exactly those found by us in the pharynx. We agree with Steinhard that the longitudinal element is much reduced or absent, giving this scale a more delicate appearance in comparison with those of the skin. Two quite marked differences were not shown by him. The lateral elements of the basal plate project distinctly anterior in these denticles and the spine arises well toward the posterior end of this scale.

Steinhard (1903), Imms (1905), and Fahrenholz (1915) found stomodeal denticles to be missing from the roof of the mouth and pharynx but present on the lining of the gill arches and the entire covering of the floor of the mouth between the level of the ventral ends of the first functional gill slits and that of the corresponding parts of the fifth pair. Cook and Neal (1921), however, reported these scales to be present not only in the above-mentioned regions but also somewhat more anterior than the first slit and, more significant, on the roof of the pharynx in small numbers. We have been unable to find denticles on the roof of the mouth or pharynx medial to the region of the dorsal ends of the gill slits. They are, however, present along inner edges of the septa and in the floor of the pharynx. We agree with Steinhard and Imms that these scales are ordinarily found ventrally, only between the first and fifth slits. Our observations differ from those of others in that we have noted that stomodeal denticles are lacking in the lateral parts of the pharyngeal floor, except for occasional ones toward the posterior ends of these regions.

Steinhard (1903) reported that comb scales occur in the pharyngeal slits both internal and external to the lamellæ. A further study verifies this statement. Certain details may be added. The areas of comb scales external to the four demibranchs on the posterior walls of the slits are considerably wider than those external to the five demibranchs of the anterior walls. Comb scales are present in considerable numbers internal to the demibranchs of posterior walls but, except for the extreme ventral and dorsal ends, they are lacking internal to the demibranchs of anterior walls. There is, of course, no demibranch on the posterior wall of the fifth cleft. The external part of this wall bears no comb scales. The internal part has many of these scales and much of the area corresponding to the positions of demibranchs of other slits has scattered scales of this type.

The spiracular cleft does not have comb scales associated with it. There are, however, stomodeal denticles. These are numerous on the posterior wall, somewhat less abundant on the anterior wall, quite limited between these regions on the dorso-medial side, and absent from the ventro-lateral wall.

From our observations, we conclude that in the integument of *Squalus* of 54 to 62 cm. length there is a quite constant pattern of distribution of scaleless areas and of scales of various types. There are at least four general types. Since three of these exist in two quite constant subtypes which do not seem to intergrade, the number of distinct types is probably about seven. In addition, there are two types which are almost certainly transitional. In the linings of the mouth, pharynx and gill slits there are two additional types—stomodeals and combs—as previously reported by Steinhard (1903) and others.

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HISTOCHEMISTRY OF THE OVARY OF FUNDULUS HETEROCLITUS WITH SPECIAL REFERENCE TO THE DIFFERENTIATING OOCYTES

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The process of differentiation in the oöcytes of many animals has been studied by histological and cytological methods. Although the general agreement in the morphological changes is very striking, the divergence in interpretation of the significance of the structures observed and the relationships between them is more so. Konopacki and Konopacka (1926), Konopacka (1935), and Guthrie (1925 and 1929) have emphasized the importance of a physiological viewpoint in such studies. The limitations of attempts to analyze the chemical contents of regions of a cell by means of their reactions with fixing or staining fluids are numerous, in spite of the fact that considerable security can be felt in some instances. With histochemical methods a somewhat different attack can be made on the question of the significance of changes in differentiating cells. Not only is the identification and specific localization of a variety of substances possible at different periods, but the use of quantitative standards of comparison gives data on the shifting concentration of materials, as Marza and Marza (1935) and Marza (1935) have shown in the hen's egg. The present collaboration was undertaken in order to provide histochemical data on a form in which cytological studies had been made, with the expectation that the sequences of differentiation in the oöcytes would be revealed more clearly.

Observations have been made on all parts of the ovary—stroma, interstitial cells, follicular theca, follicular epithelium, zona radiata, and oöcytes in all stages of differentiation. For purposes of reference in the descriptions the differentiating oöcytes are placed in two main groups (Fig. 1). Those in the first part of the growth-period (Period 1) have increasing amounts of cytoplasm, with cytosomes that may range up to 300 microns in diameter, but no yolk vesicles (Figs. 8, 12, and 16).

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[1] Dr. and Mrs. Marza have made all of the histochemical analyses recorded in this paper. Their report has been prepared for publication by Mary J. Guthrie, who provided the specimens, through the Supply Department of the Marine Biological Laboratory, at Woods Hole, Massachusetts, U. S. A., and who has correlated the histochemical observations with cytological observations on samples from the same and many other specimens.

Period 2 begins with the appearance of vesicles (proteinaceous yolk vesicles of Guthrie, 1928) in the oöcytes and includes the remainder of the period of differentiation. During stage A_1 of the second period there are few yolk vesicles and the diameter of the cell may be as great as 400 microns (Figs. 16, 17, and 18). The increase in number of yolk vesicles distinguishes stage A_2, at the end of which only a narrow zone of peripheral cytoplasm is free of vesicles and cells may be 600 microns in diameter (Fig. 14). Between the vesicles the internal cytoplasm is seen as a reticulum. The zona radiata appears at this stage, and the nucleus of the oöcyte is increasingly eccentric. The beginning of stage B_1 is marked by the initial appearance of globules of yolk (intravesicular yolk) within the vesicles (Figs. 10 and 13). During stage

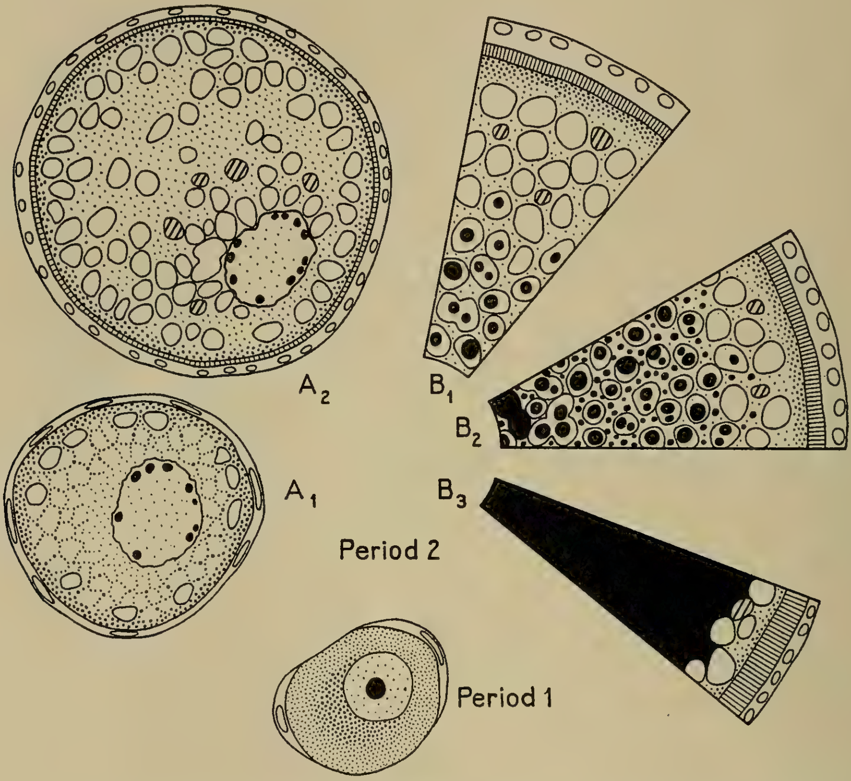

FIG. 1. Diagram showing the stages in the growth-period of oöcytes in *Fundulus* which are referred to throughout the text. In the cytosome the circles which are empty represent the vesicles in which yolk may appear later; circles which are lined represent fat deposits; and circles which are solid represent either intra- or extravesicular yolk.

B_2 globules of extravesicular yolk begin to appear directly in the cytoplasm between the vesicles, and the diameter of the oöcyte may reach 1,000 microns (Fig. 15). The globules of yolk that appear within the so-called proteinaceous yolk vesicles, as well as those which appear between the vesicles, constitute the fatty yolk of Guthrie (1928). As the oöcyte continues to grow, intravesicular and extravesicular yolk increases in amount. The yolk globules deposited within and without the original large vesicles become confluent and indistinguishable (Fig. 13). Toward the end of the growth-period (stage B_3), a continuous mass of yolk is found surrounded by a cortical layer of cytoplasm (Figs. 11, 12, and 13) in which the germinal vesicle is located. Primary oöcytes may reach 1,600 microns in diameter.

This report includes observations on the variations in amount and distribution of iron and potassium, on the plasmal and nucleal reactions, and on the localization and relations of the acid proteins. Other analyses are in progress.

Determination of Iron

Iron occurs in fish eggs in the ichthulin molecule which, like vitellin, contains a hematogen (Walter, 1891, in the carp). A histochemical study of iron has been made by Smiechowski (1892), Wassermann (1910), Marza, Marza, and Chiosa (1932), and Marza (1935) in the hen's egg at various stages in its growth. In *Fundulus heteroclitus* we have studied the localization and changes in content of iron in the oöcytes during the period of growth and yolk-formation.

The ovaries used were from fish collected at Woods Hole, Massachusetts, on June 27 and September 11, 1934. Fixation was in 96 per cent alcohol, and paraffin sections 10 microns in thickness were studied. Since it seemed probable that both inorganic and organic iron were present in eggs (*cf.* Warburg, 1914, on eggs of *Strongylocentrotus*), methods for detecting inorganic and total iron content were used. Macallum's (1912) ammonium-sulfide method and Liesegang's (1923) potassium-ferrocyanide method were employed to test for inorganic iron, while Policard's (1924) microincineration method made possible the identification and localization of the total iron content.

With Macallum's method certain granules in the stroma, follicle, and oöcyte become dark brown, which presumably is evidence of their inorganic iron content. However, some of these dark brown granules are apparently pigment since they are seen in similar locations after the use of Liesegang's method, with which the presence of inorganic iron is indicated by a homogeneous blue staining of certain regions. It appears, therefore, that Macallum's method is not entirely trustworthy for this material. Tests for inorganic iron have not been considered positive unless the results with the two methods were in agreement. After the incineration of sections according to the method of Policard the various parts of the ovary are recognizable. Of the mineral residues only the oxides of iron are colored orange or red; others are white or gray. The detection of iron and its localization within the cells are

TABLE I

Results of tests for inorganic iron and total iron content. Four hundred and twenty-eight oöcytes in 12 ovaries were examined. A (?) indicates that a result is considered unreliable for reasons stated in the text.

Region	Methods		
	Macallum	Liesegang	Policard
Interstitial cells	+	+	+
Stroma	+ (?)	–	–
Theca			
Period 2 A	+	+	– (?)
Period 2 B	+	+	– (?)
Follicular epithelium			
Period 1	–	–	–
Period 2 A	+	+	+
Period 2 B	+	+	+
Zona radiata			
Period 2 A	+	+	–
Period 2 B	+	+	–
Oöcyte cytoplasm			
Period 1	–	–	–
Peripheral region			
Period 2 A	+	+	– (?)
Period 2 B	+	+	– (?)
Internal region			
Period 2 A	+	+	+
Period 2 B	+	+	– (?)
Yolk			
Intravesicular	+	+	+
Extravesicular	–	–	+
Oöcyte nucleus			
Chromatin	–	–	–
Nucleoli	+ (?)	–	–

thus possible. Microincinerated sections were examined against a dark field with a Greenough microscope and, also, by means of a Reichert oblique illuminator.

Inorganic iron is most abundant in the interstitial cells, some of which give a more intense reaction than others, and is rarely seen in the stroma. The nucleus of the growing oöcyte does not contain iron at any stage. During the first part of the growth-period iron has not been demonstrated either in the cells of the follicle or in the cytoplasm of the oöcyte. However, in the second period inorganic iron is sometimes seen in the follicle cells and in the zona radiata but not throughout the entire circumference of any one egg. Within the oöcyte at this stage a positive inorganic iron test of weak intensity is sometimes obtained in the peripheral layer of cytoplasm, as well as in the internal cytoplasm between the yolk vesicles. In the intravesicular yolk the inorganic iron test is sometimes positive, but in the extravesicular yolk inorganic iron has not been detected. After microincineration the fully differentiated yolk is seen to contain iron, which must have been in organic combination (Fig. 11). The summary of tests for iron is given in Table I.

It should also be recorded that white ash (probably calcium) is abundant in the cytoplasm of oöcytes in Period 1 (Fig. 8). These oöcytes differ strikingly from those of the hen, which do not contain ash at a comparable stage. White ash also occurs in both the peripheral and internal cytoplasmic regions during Period 2 in oöcytes of *Fundulus* (Fig. 9). This white ash may obscure the red where it is present in small amounts and render the negative microincineration results for iron unreliable for certain regions. As the intravesicular yolk appears a gray ash remains after microincineration (Fig. 10). The nucleoli of the fish egg are very rich in white ash, and the chromatin in gray ash.

Inorganic iron apparently passes through the follicle and zona radiata of the oöcyte during the period of yolk deposition and is found in the cytoplasm and in the yolk forming within the vesicles. During the final differentiation of yolk the accumulated iron must be combined in organic form, probably in the hematogen of ichthulin. The finding of iron in both inorganic and organic form may account for certain discrepancies in previous reports.

Quantitative Determination of Potassium

The quantity of potassium exceeds the combined amounts of calcium, sodium, and magnesium in ripe fish eggs. Bialaszewicz (1929) has shown that high potassium content is characteristic of egg cytoplasm of both invertebrates and vertebrates. Seventy-four to 92 per cent of all the diffusible bases in egg cytoplasm is potassium. Chemically the potassium content of a number of ripe and fertilized fish eggs has been determined (Needham, 1931, p. 356), but the eggs of *Fundulus* have not been studied from this point of view. A histochemical study of potassium has not been made on any fish egg, nor have studies been made on any differentiating oöcytes.

The method used was that of Macallum (1905) as modified by Marza and Chiosa in 1934 and 1935. By means of a comparison eyepiece and a series of standards made of agar-agar and containing concentrations of cobalt sulphide calculated as potassium equivalents, it is possible to determine the quantity of potassium in specific regions of the ovaries (Fig. 2).[2] Ovaries from twelve fish were fixed in 96 per cent alcohol and sectioned in paraffin at 10 microns. One hundred and thirty-six oöcytes of Period 1, 63 of Period 2A, and 72 of Period 2B

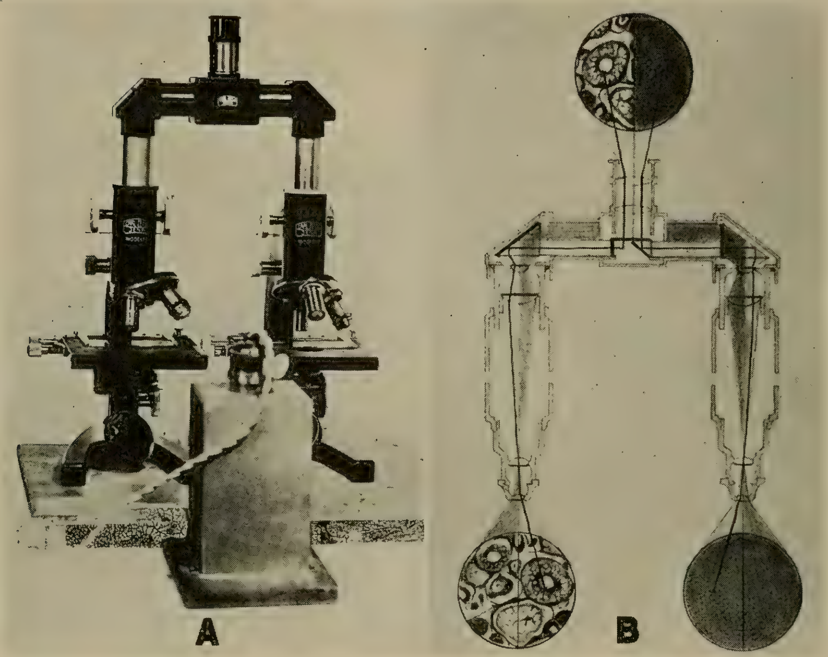

FIG. 2. Use of comparison eyepiece in quantitative histochemical observations. *A*, arrangement of microscopes and light; *B*, diagram of preparation of ovary (left, below), standard slide (right, below), and field of vision (center, above).

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[2] Marza and Chiosa (1935) have given the technique for the preparation and use of the standard slides.

have been studied. Within these stages of differentiation the oöcytes observed have been sorted according to actual size attained.

The smallest oöcytes contain more potassium in their cytosomes than do larger ones (Figs. 3 and 12, and Table II). Since these cells, which are from 20 to 50 microns in diameter, do not have a continuous follicular layer the concentration of potassium is not conditioned by selective permeability of the follicle (Fig. 1). The nucleoli of such young oöcytes are very rich in potassium but the chromatin contains little.

Table II

Results of tests for potassium, indicated as average percentages. Two hundred and seventy-one oöcytes in 12 ovaries were examined.

Region	Percentage K	Region	Percentage K
Stroma		Oöcyte cytoplasm	
Cytoplasm	0.065	Period 1	0.160
Nucleus	0.130	Peripheral region	
		Period 2 A	0.087
Theca		Period 2 B	0.084
Cytoplasm		Internal region	
Period 2 A and B	0.032	Period 2 A	0.087
Nucleus		Period 2 B	0.109
Period 2 A and B	0.087		
Follicular epithelium		Yolk	
Cytoplasm		Intravesicular	0.108
Period 1	—	Extravesicular	0.075
Period 2 A	0.066		
Period 2 B	0.057	Oöcyte nucleus	
Nucleus		Chromatin	
Period 1	—	Period 1	0.098
Period 2 A	0.109	Period 2 A	0.049
Period 2 B	0.116	Period 2 B	0.065
		Nucleoli	
Zona radiata		Period 1	0.151
Period 2 A	0.054	Period 2 A	0.125
Period 2 B	0.057	Period 2 B	0.130

During the second period the theca is somewhat more conspicuous, and the concentration of potassium is not the same in it as in the cells of the stroma (Table II). In the follicle cells the nuclei are richer in potassium than are the cytosomes. No concentration of potassium occurs in the zona radiata. With the appearance of vesicles in the oöcyte (Period 2 A_1) the concentration of potassium in the cytoplasm continues to decrease (Fig. 3). The penetration of potassium does not keep pace with the rate of cytoplasmic increase in the oöcyte. This decrease is noticeable, also, in the chromatin and nucleoli. During the early stages of yolk deposition the percentage of potassium in the cytoplasm begins to rise, especially in the internal region. Yolk contains a high percentage of potassium which again falls off as the oöcyte grows

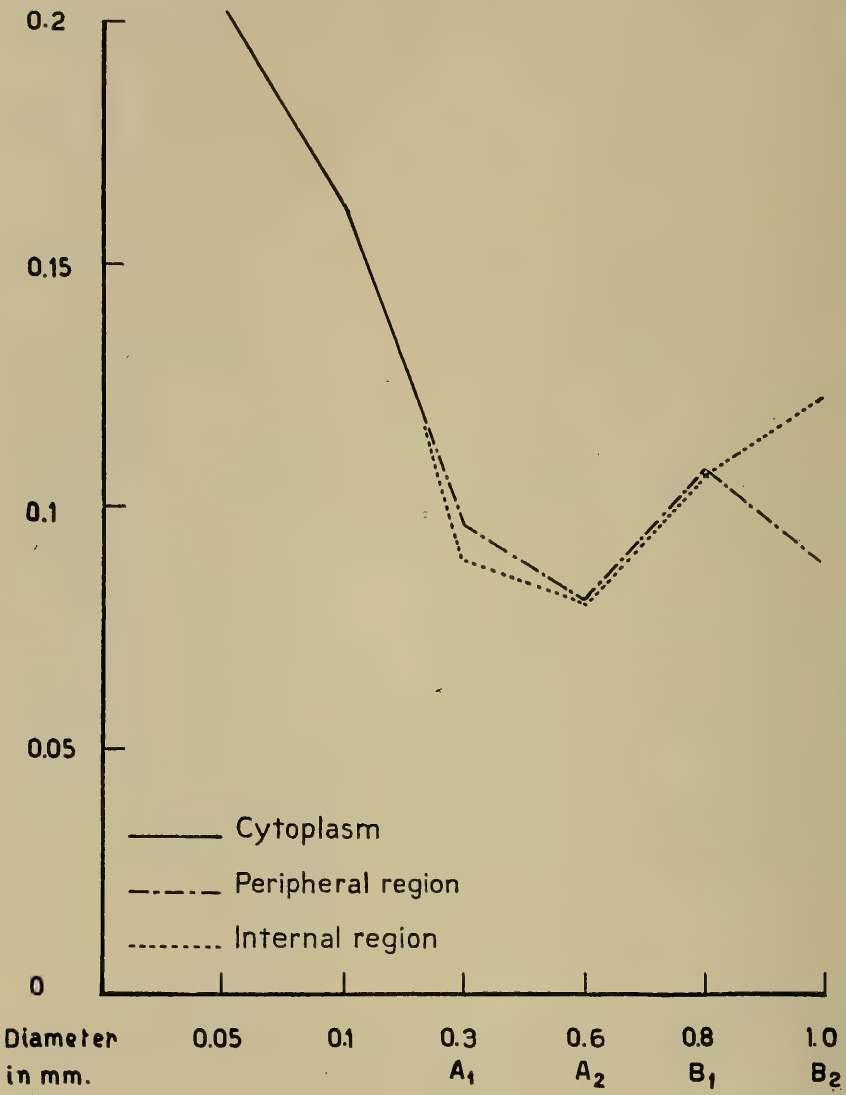

FIG. 3. Variation in percentage of potassium, shown on vertical axis, in cytoplasm of oöcytes during the growth-period.

(Fig. 12). A similar shift in proportion of potassium during yolk-formation has been observed in the hen's egg in the ground substance of both white and yellow yolk-spheres.

The fact that the theca and follicular epithelium vary so little with respect to potassium content throughout the growth-period is different from the findings in the ovary of the hen (Marza and Chiosa, 1936). There, toward the end of yolk-formation, the percentage of potassium increases in the follicle cells. In the ovary of the fish there appears to be little increased concentration, or temporary storage, in the follicle cells of substances utilized in the formation of yolk. However, there is some indication that the follicle cells determine the rate of penetration of potassium which decreases in amount in the cytoplasm of the oöcyte following the association of follicle cells. The follicular epithelium apparently undergoes changes in permeability. It seems to be more permeable to potassium during stages A_2 and B_1 and only slightly permeable at later stages. That the result may be conditioned by an altered rate of entrance of other materials is also a possibility.

Plasmal Reaction

In 1924 two color reactions were reported for cells after the application of Schiff's sulfurous-fuchsin reagent. The first or plasmal reaction was observed in the cytosome, and the nuclei were uncolored except when the reagent was applied for a long period (Feulgen and Voit, 1924). If the sections were hydrolyzed with hydrochloric acid before being treated with Schiff's reagent, the color reaction was in the nucleus; this was called the nucleal reaction (Feulgen and Rossenbeck, 1924).

The names given to these reactions indicate that aldehydes in the cytoplasm or chromatin were believed to be responsible for the appearance of the color. Feulgen and Voit (1924) pointed out that the plasmal reaction was related to the presence of fat in the cytoplasm. If the tissue is dehydrated before sectioning the reaction is usually negative. Verne (1929) demonstrated that only unsaturated fats in the process of oxidation gave a positive plasmal reaction. However, fatty substances are not the only ones to react positively. Feulgen and Voit (1924), Voss (1929), and Marza and Marza (1934) have reported an intense plasmal reaction in the elastic elements of large blood vessels, and it occurs in tissue dehydrated after fixation. Many who use this method of Feulgen consider it to be specific for aldehydes. Feulgen and co-workers pointed out that while Schiff's reagent reacted with alkalis (a red color) and bromine they considered it to be specific for aldehydes in cells. Lison (1932) found that the color of the reaction with various aldehydes varied from deep violet to blood red. He concluded, therefore, that similar color reactions by substances other than aldehydes should be considered as positive. With this criterion alkalis, aliphatic ketones, certain unsaturated compounds (such as oleic acid), weak salts of strong bases (such as acetates and phosphates), some amino oxides, and certain catalytic oxidizing systems gave positive color tests. Even though certain of these substances occur not at all or in very minute quantity in normal cells, the presence of alkalis, phosphates, ketones, and oxidases makes it clear that a positive plasmal reaction cannot be considered specific for aldehydes or fats.

TABLE III

Results of plasmal and nucleal reactions, indicated as averages of color intensity; 0.3 is the lowest positive reading.

Region	Plasmal reaction	Nucleal reaction
Stroma		
Cytoplasm	—	—
Nucleus	—	1.25
Theca		
Cytoplasm	—	—
Nucleus	—	1.25
Follicular epithelium		
Cytoplasm	—	—
Chromatin	—	1.25
Nucleoli	—	1.25
Nucleoplasm	—	0.3
Zona radiata	0.3	0.3
Oöcyte cytoplasm		
Period 1	0.3	0.4
Peripheral region		
Period 2 A and B	0.3	0.3
Internal region		
Period 2 A and B	0.3	0.3
Yolk		
Intravesicular	0.75	0.75
Extravesicular	0.6	0.5
Oöcyte nucleus		
Period 1	—	—
Period 2 A	—	—

Voss (1927) first studied the plasmal reaction during the growth-period of oöcytes, using ovaries of amphibians. Hibbard (1928) and Brachet (1929) employed it with amphibian ovaries, and Marza and Marza (1934) with the hen's ovary. For the reaction in *Fundulus,* twelve ovaries were fixed in sublimate-acetic as recommended by Feulgen. Frozen sections were used in order not to lose the fatty sub-

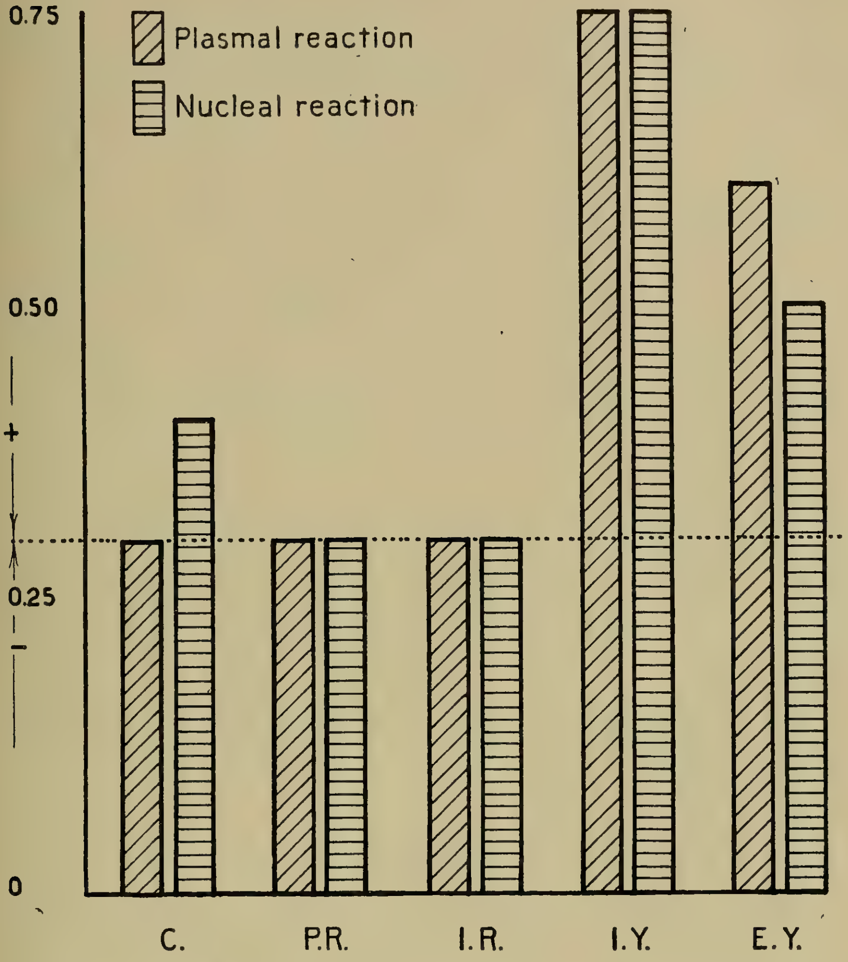

FIG. 4. Plasmal and nucleal reactions in the oöcytes. *C,* cytoplasm of oöcytes of Period 1; *P. R.,* peripheral region of cytoplasm of oöcytes of Period 2; *I. R.,* internal region of cytoplasm of oöcytes of Period 2; *I. Y.,* intravesicular yolk; and *E. Y.,* extravesicular yolk. The numbers on the vertical axis refer to the comparison color scale.

stances during dehydration. The sulfurous-fuchsin reagent was prepared according to Wermel (1927),[3] and the short technique was followed: thirty minutes in the reagent, rapid washing in three jars of water turbid with sulfur dioxide, washing in distilled water, dehydration, and mounting. In order to evaluate the reaction qualitatively a scale of color intensity was employed. An intensity designated as 0.3 indicates the least positive reaction and below that value the coloring is the result of adsorption of the reagent; 3.0 is an extremely intense positive reaction.

A slightly positive plasmal reaction may be obtained in the cytoplasm of oöcytes of Period 1 (Table III and Fig. 4). In this material the so-called yolk-nucleus, as described by Hibbard (1928) in *Discoglossus* and Marza and Marza (1934) in the hen, is not distinguishable. The reaction of the nucleus of the oöcyte is negative at all times except for an occasional faint reaction in a nucleolus. No reaction is observed in the ovarian stroma, the theca, or the follicular epithelium at any stage. The interstitial cells give an intense positive reaction.

During the second period the zona radiata varies in reaction, apparently depending on the material present in its canals at any particular time; it is positive in about half the cases. In the peripheral and internal cytoplasm of the oöcyte the reaction is variable with an average on the positive side. Yolk forming within the vesicles gives a more positive reaction than that formed directly in the cytoplasm. Since the reaction is the same in sections dehydrated in alcohol before being treated with the Schiff's reagent, the substance responsible cannot be fat. The difference in degree of reaction in the intravesicular and extravesicular yolk parallels the difference in potassium content. It seems possible that the plasmal reaction in the yolk is conditioned by the presence of the alkali.

NUCLEAL REACTION

The nucleal reaction has been studied in the eggs of numerous invertebrates and some vertebrates. Contrary to the usual positive reaction in the nuclei of cells, the nucleal reaction becomes negative in the nuclei of oöcytes early in the growth-period and remains negative until the period of diakinesis (Koch, 1925; Voss, 1927; Hibbard, 1928; Ludford, 1928; Brachet, 1929; Gresson, 1930; Mukerji, 1930; Bauer, 1932;

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[3] Dissolve 1 gram of basic fuchsin in 200 cc. of boiling distilled water and filter. (Grübler's gelblich fuchsin was used.) Add 20 cc. of normal hydrochloric acid. When the solution is cool add 2 grams sodium bisulfite and stir for several minutes. After 2 hours add 0.2 cc. acetaldehyde; the solution becomes intensely red. After 45 minutes again add 20 cc. of normal hydrochloric acid and 1 gram of sodium bisulfite. Stir the solution for 15 minutes and set aside until the fuchsin is decolorized. The pale amber reagent should be kept dark and cool.

Marza and Marza, 1934). This peculiar situation is characteristic of only female germ cells, and the nucleal reaction is positive during the entire course of spermatogenesis.

Ovaries of *Fundulus* were fixed in sublimate-acetic, and the Feulgen-Rossenbeck technique was followed on paraffin sections. The color scale used for the plasmal reaction records was again employed.

The cytoplasm of cells of the stroma and of the follicular epithelium has a negative nucleal reaction (Table III). The nuclear membrane and chromatin, but not the nucleoplasm, of cells of the stroma give positive tests. In the nuclei of the follicular epithelium the reaction is negative in the membrane, faintly positive in the nucleoplasm, and equally strong in the chromatin and in the nucleoli. These reactions do not change conspicuously during the course of differentiation.

There is a positive reaction in the cytoplasm of the oöcyte during the first period of differentiation which is similar to the reaction without hydrolysis—the plasmal reaction (Fig. 4). It seems likely that this is to be explained on the basis of the potassium content. Even in the smallest oöcytes the nucleal reaction is negative in all parts of the nucleus.

During the second period of differentiation the zona radiata has a faintly positive reaction in stage B. The cytoplasm at the periphery of the oöcyte and between the yolk globules gives a faintly positive reaction. As with the plasmal tests, the yolk gives a positive nucleal reaction of similar intensity. Since hydrolysis does not alter the reaction, it is apparent that it is not always conditioned by fat, and that it may be attributed to the potassium content. In the oöcyte of the hen Marza and Marza (1934) made further tests that led them to the hypothesis that the positive plasmal and nucleal reactions arise from the presence of potassium. They found in the yolk of the hen's egg that the plasmal and nucleal reactions were localized in the centers of the globules. When the nucleoprotein test (method of Unna, 1921) was positive the localization was peripheral. The reaction for oxidases was negative. Prolonged extraction with ether and chloroform did not alter the reaction, which eliminated fats as the source of aldehyde. There was no augmentation of the reaction after treatment with alcohol; it had been suggested that alcohol might be adsorbed by the yolk and partially changed to aldehyde.

The nucleal reaction is negative in nuclei of all oöcytes during the second period in *Fundulus,* and no observations were made on the time of shift of the reaction before meiosis. There is no evidence upon which to offer an explanation of this negative test. Koch (1925) and Brachet (1933) attributed it to a chemical change in the nucleic acid during the growth-period, while Feulgen (1926) and Ludford (1928) considered it to be the effect of dispersion or dilution of thymonucleic acid as the nucleus of the oöcyte increased in volume.

Acid Proteins

In order to study changes in the proteins during differentiation of the oöcyte the method of Unna (1921) for acid proteins was used. This procedure enables one to distinguish albumins, globulins, and nucleoproteins. It is based on differences in color reaction and solubility between the groups. Tissues are fixed in 96 per cent alcohol, sectioned in paraffin, and stained for twenty minutes in the Pappenheim-Unna reagent.[4] Albumins and globulins are stained red by the pyronin, while the nucleoproteins are stained blue-green with the methyl green. To separate the albumins and globulins advantage is taken of their specific solubilities. Albumins and pseudoglobulins are soluble in distilled water while globulins are not. Both albumins and globulins are soluble in salt solutions. By comparison of sections stained before and after chromolysis in water and in salt solution the location and relative proportions of acid proteins of the three groups can be determined.

Sections of the ovary of *Fundulus* were divided into three series. One series was stained directly. In such sections all acid proteins were shown. A second series was placed in sterile distilled water in an oven at 39° C. for twenty-four hours before staining. This was the first chromolysis. The difference in reaction with pyronine between these sections and those of the first series was conditioned by the loss of albumins (*cf.* Figs. 16 and 18). A third series was placed in a sterile 2 per cent solution of sodium chloride in an oven at 39° C. for twenty-four to forty-eight hours before staining. This was the second chromolysis which removed both albumins and globulins. If nucleoproteins were present in such sections they would then be stained with the methyl green (*cf.* Fig. 17 with Figs. 16 and 18). To obtain results subject to comparison, sections of the three series were handled simultaneously by an arrangement of forceps during the staining and dehydration. The latter process had to be completed rapidly. Results were read on a standard color scale in which 0.3 was the least positive reaction and 4.0 the most intense. Two hundred and sixteen oöcytes have been studied in sections from twelve ovaries.

Two varieties of interstitial cells are demonstrated with Unna's technique. Those which are pigmented, occur in masses, and contain iron do not color with the stains used. Cells of the other type have variable shapes, with pseudopodia-like histiocytes or simple elongations like fibroblasts, or may be oval with eccentric nuclei somewhat similar

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[4] The Pappenheim-Unna reagent used consisted of 0.15 gram of Grübler's methyl green (from which admixed methyl violet was removed by shaking the crystals in chloroform), 0.25 gram of pyronine, 2.5 cc. of 96 per cent alcohol, and 100 cc. of 0.5 per cent phenol.

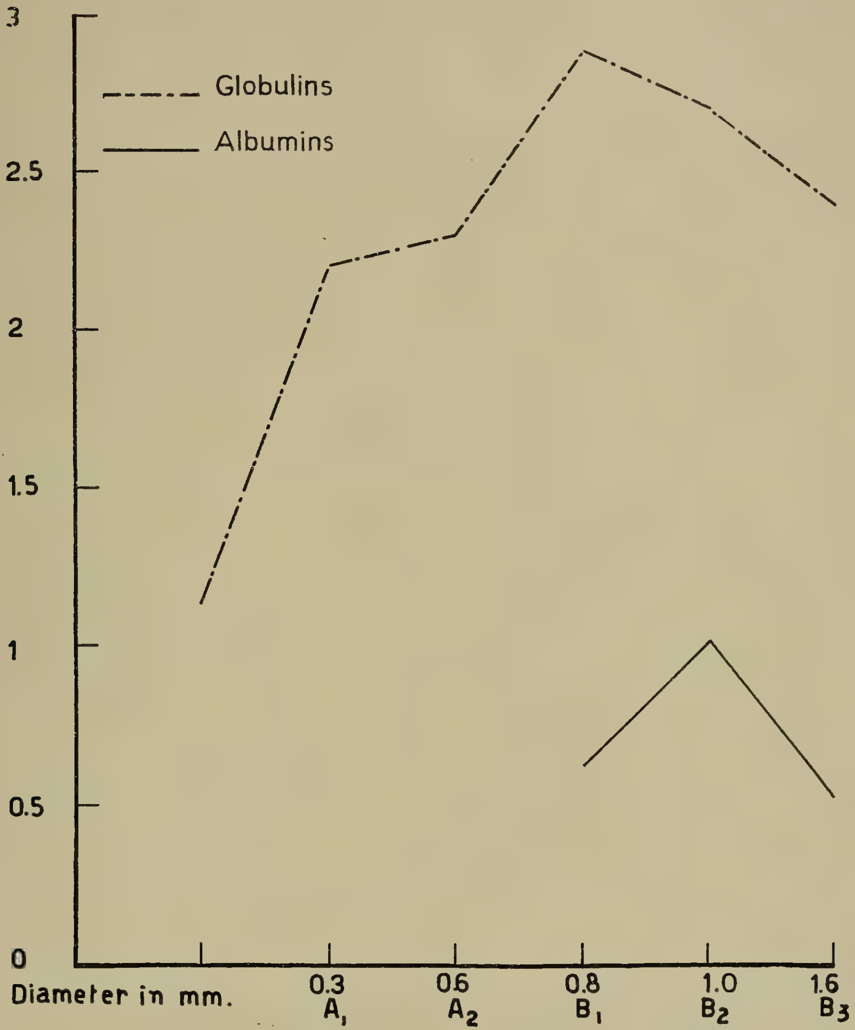


FIG. 5. Variation in acid proteins in the cytoplasm of follicle cells. The numbers on the vertical axis refer to the comparison color scale.

to plasmocytes. Such cells have been described in the ovarian stroma of the hen by Goodale (1919), Nonidez (1921), Marza (1934), and Marza and Golaescu (1935); the analogies are not completely clear.

These cells in *Fundulus* are found in the endothelium of the blood vessels, isolated in the stroma, in the tunica albuginea, and, rarely, within the masses of pigmented cells. The cytoplasm of the non-pigmented interstitial cells stains intensely with the pyronine.

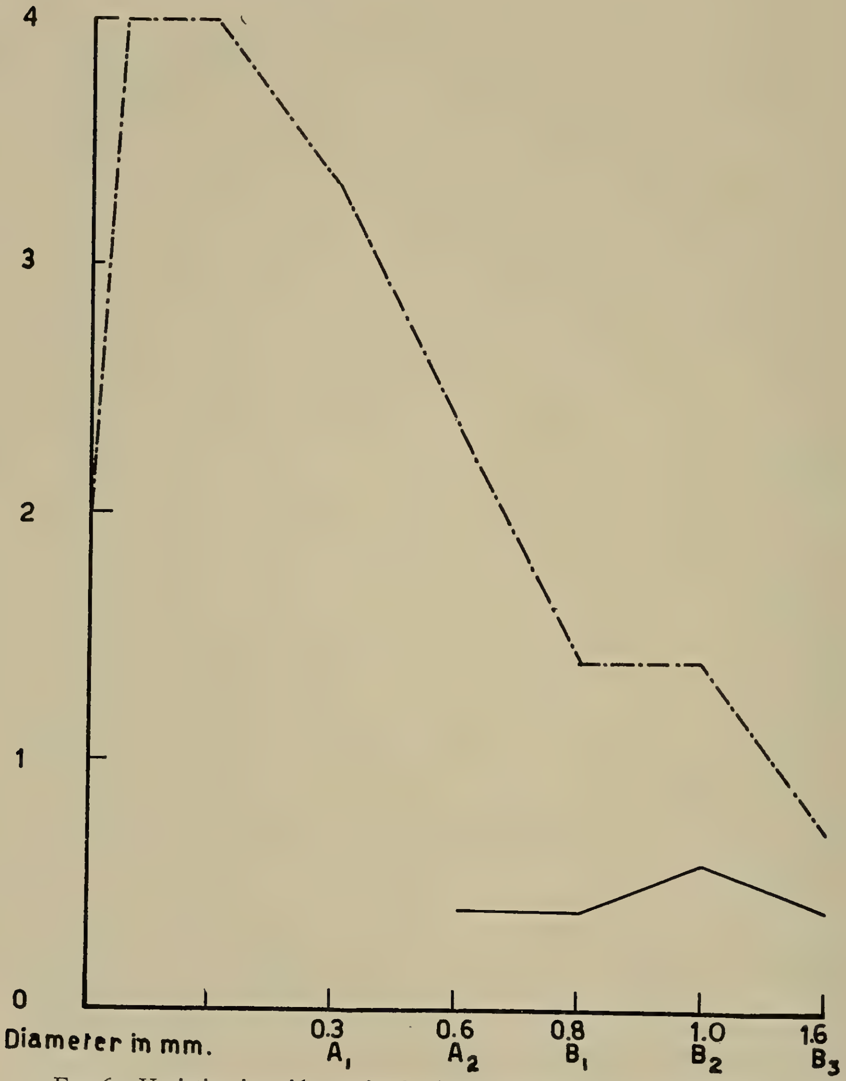


FIG. 6. Variation in acid proteins in the cytoplasm of oöcytes in Period 1 and in the peripheral region of cytoplasm in Period 2. Designations as in Fig. 5.

Only a small quantity of irregularly localized globulin is found in the cytoplasm of cells of the ovarian stroma, and neither globulins nor albumins occur in the nuclei which are, however, rich in nucleoproteins (Table IV). Both globulins and albumins are found in the cytoplasm of thecal cells during Period 2A; the amount is somewhat reduced during Period 2 B. The nuclei of thecal cells are rich in nucleoproteins. Concentrated masses of globulins are seen localized along the length of the cytosomes of follicle cells during Period 1 (Fig. 5), but the ground

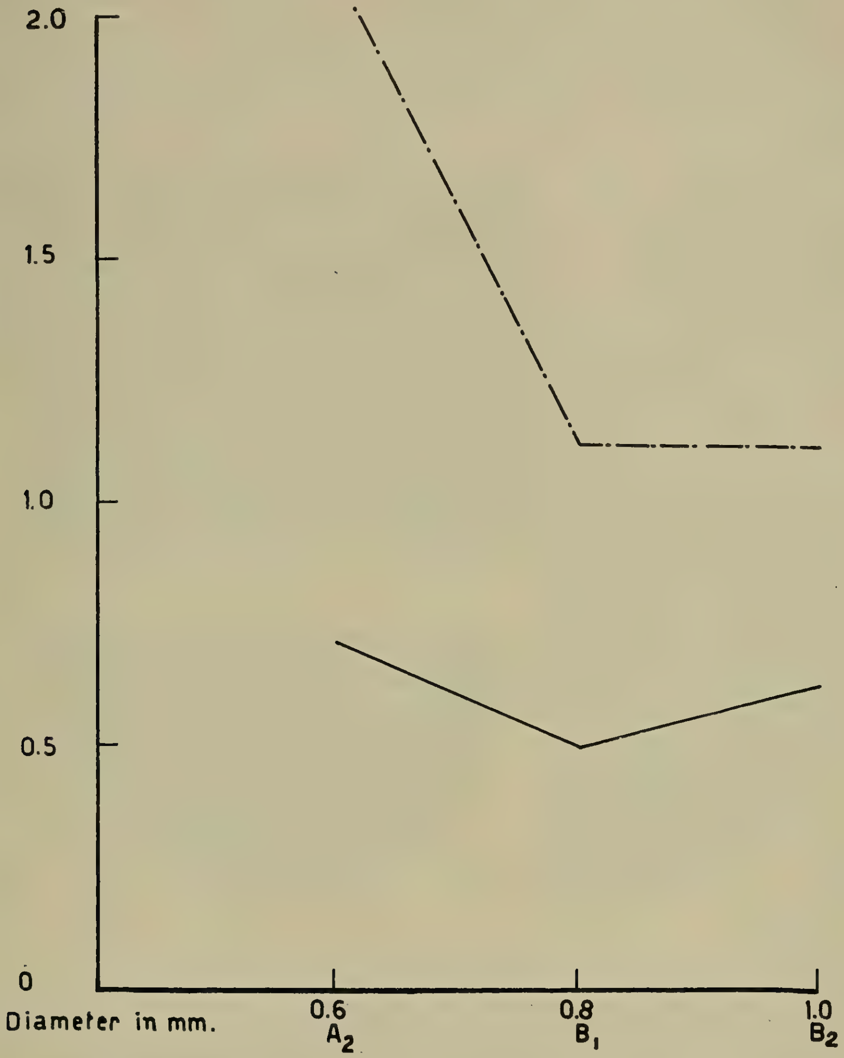
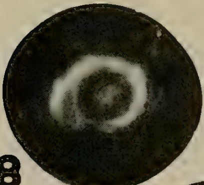


FIG. 7. Variations in acid proteins in the internal regions of cytoplasm in oöcytes of Period 2. Designations as in Fig. 5.

cytoplasm of follicle cells gives only a slight reaction (0.5). The amount of globulins in the follicle is increased conspicuously during Period 2, and albumins appear in stage 2B. These albumins may occur because of the splitting of globulins in the follicle as penetration into the oöcyte begins. Globulins appear in the chromatin during stage 2 B and are found, together with nucleoproteins, in the nucleoli throughout the growth-period. No acid proteins have been detected in the zona radiata at any time.



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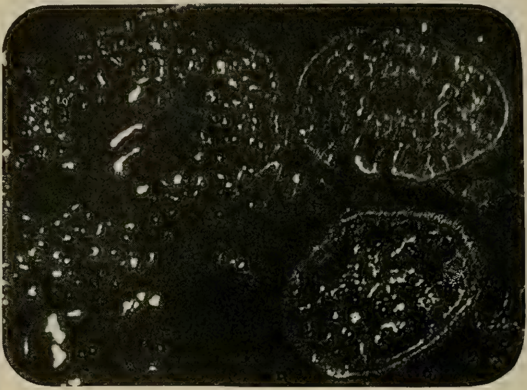

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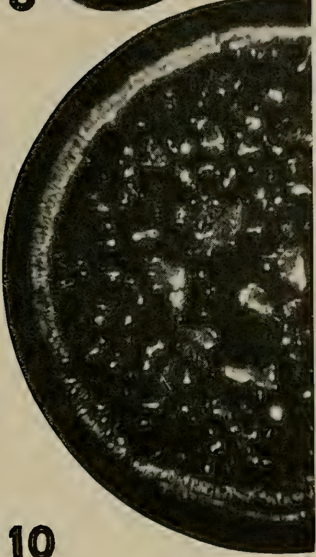

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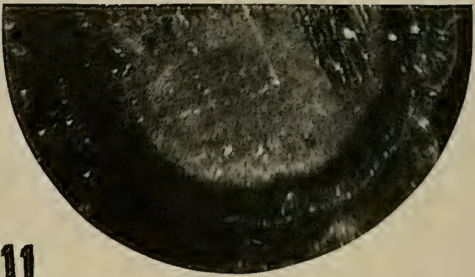

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Explanation of Plate I

Microphotographs

Fig. 8. Small oöcyte (Period 1) after microincineration, showing much white ash in cytosome and some in nucleus. × 150.

Fig. 9. Oöcytes of Period 2 A after microincineration. Ash is visible in the cytoplasm but not in the vesicles. × 30.

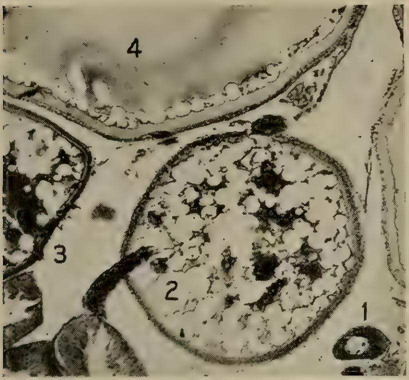
Fig. 10. Oöcyte of Period 2 B_1 after microincineration. White ash occurs in the region of the cytoplasm and gray ash in the intravesicular yolk which is present. The follicle appears two-layered because of the plane of the section. × 56.

Fig. 11. Oöcyte of Period 2 B_3 after microincineration. The homogeneous central mass of yolk is somewhat shrunken from the peripheral cytoplasm and its ash content is visible. × 30.

TABLE IV

Results of tests for acid proteins, indicated as averages of color intensity; 0.3 is the lowest positive reading. Where a range is given it indicates a shift in content between the smaller and larger cells of a given period.

Regions	Albumins	Globulins	Nucleoproteins
Stroma			
Cytoplasm	—	0.8	—
Chromatin	—	—	2.5
Nucleoli	—	—	3.0
Theca			
Cytoplasm			
Period 2 A	0.4	0.6	—
Period 2 B	—	0.6	—
Chromatin			
Period 2 A and B	—	—	2.0
Follicular epithelium			
Cytoplasm			
Period 1	—	1.1	—
Period 2 A_1–A_2	—	2.2–2.3	—
Period 2 B_1	0.6	2.9	—
Period 2 B_2–B_3	1.0–0.5	2.7–2.4	—
Chromatin			
Period 1	—	—	2.5
Period 2 A	—	—	3.0
Period 2 B	—	3.0	3.0
Nucleoli			
Periods 1 and 2	—	3.0	3.0
Oöcyte cytoplasm			
Period 1	—	2.0–4.0	—
Peripheral region			
Period 2 A_1	—	3.4	—
Period 2 A_2	0.4	2.4	—
Period 2 B_1	0.4	1.4	—
Period 2 B_2–B_3	0.6–0.4	1.4–0.7	—
Internal region			
Period 2 A_2	0.7	2.4	—
Period 2 B_1	0.5	1.1	—
Period 2 B_2	0.6	1.1	—
Yolk			
Intravesicular			
Period 2 B_1	—	0.75	0.5
Period 2 B_2	—	0.5	0.5
Extravesicular			
Period 2 B_2	—	—	0.5
Oöcyte nucleus			
Chromatin			
Periods 1 and 2	0.3	0.5	—
Nucleoli			
Periods 1 and 2	—	3.5	—



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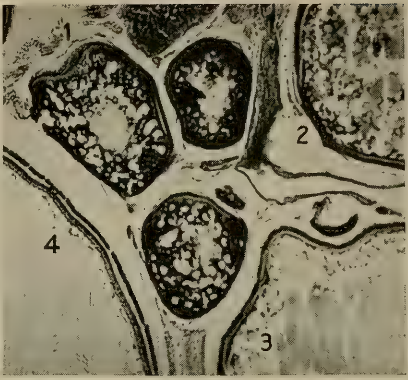

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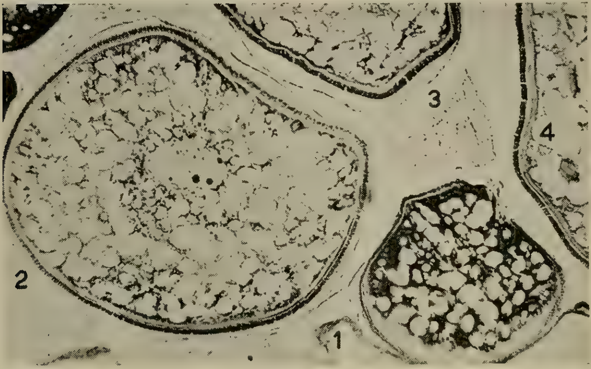

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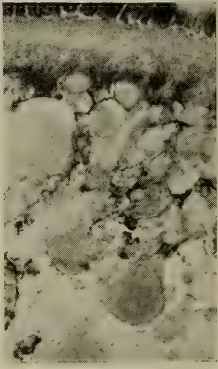

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Explanation of Plate II

Microphotographs

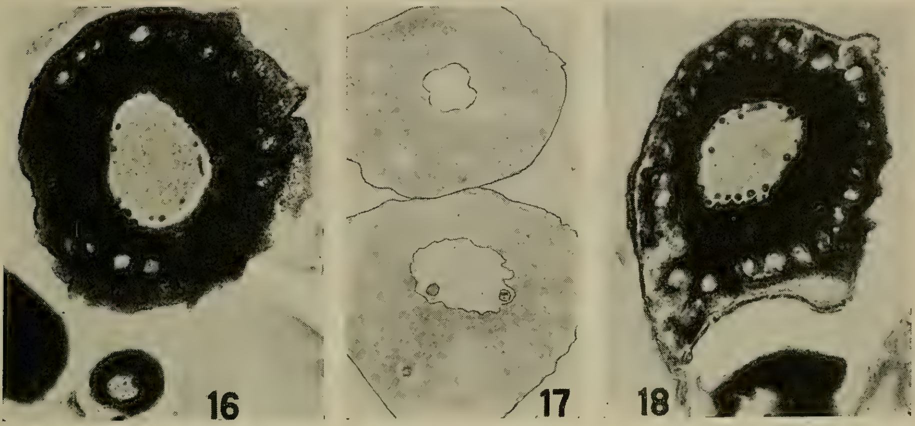
Fig. 12. Group of oöcytes, showing variation in potassium content; method of Macallum. × 41. Oöcyte 1 is in Period 1 and has a high potassium content. Oöcyte 2 is in Period 2 B_1; 3 is in Period 2 B_2; and 4 is in Period 2 B_3 (4 is about 1,200 microns in diameter). Note the decrease in potassium content in the yolk in oöcytes 3 and 4.

Fig. 13. Group of oöcytes showing variation in total acid proteins; method of Unna, without chromolysis. × 17. Oöcyte 1 is in Period 2 A_2 and gives an intense reaction in the cytoplasm; oöcyte 2 is in Period 2 B_1; 3 is in Period 2 B_2; and 4 is in Period 2 B_3 with a central homogeneous mass of yolk (4 is about 1,200 microns in diameter). Note shift in reaction in yolk.

Fig. 14. Group of oöcytes showing variation in total acid proteins; method of Unna, without chromolysis. × 41. Oöcytes 1, 2, and 3 are in Period 2 A_2 with numerous vesicles; and 4 is in Period 2 B_2. Note shift in reaction in cytoplasm.

Fig. 15. Portion of oöcyte early in Period 2 B_2 showing variation in acid proteins; method of Unna, without chromolysis. × 204. Note larger heterogeneous vesicles containing some intravesicular yolk and the much smaller globules of extravesicular yolk.

Acid proteins with a globulin reaction are conspicuous in the cytoplasm of the smallest oöcytes and reach a very high concentration by the end of Period 1 (Fig. 6). They are uniformly distributed in the cytosome (Figs. 16 and 18). No albumins or nucleoproteins are observed at this stage (Table IV). As vesicles appear and increase in number the peripheral layer of cytoplasm has a decreasing amount of globulins (Figs. 6, 13, 14, and 15). Again we see in the oöcyte of *Fundulus* during the first period a conspicuous accumulation of substances before the actual synthesis of yolk begins. Albumins appear toward the end of stage 2 A and are found in small quantities until the end of yolk-formation (Fig. 6). In general there is less acid protein in the internal cytoplasm, but the albumins are somewhat more abundant (Fig. 7). It would appear as if proteins adjacent to the places of yolk deposition were chiefly in the form of simple albumins. However, in the yolk deposited within the vesicles some globulins are found but no albumins, while in extravesicular yolk neither can be demonstrated (Fig. 15). The synthesis of simple acid proteins into ichthulin and its combination with other groupings is apparently complete and not de-



EXPLANATION OF PLATE III

Microphotographs

FIG. 16. Oöcytes showing intense acid protein reaction; method of Unna, without chromolysis. × 140. Smaller oöcyte is in Period 1, larger is in Period 2 A_1.

FIG. 17. Oöcytes in Period 2 A_1, showing the absence of globulins; method of Unna, after chromolysis in salt solution. × 140. The failure to stain with methyl green indicates the absence of nucleoproteins.

FIG. 18. Oöcytes showing globulin content; method of Unna, after chromolysis in water. × 140. Smaller oöcyte, of which only a part is seen, is in Period 1; larger is in Period 2 A_1.

stroyed by the method of treatment. This differs from the findings in the hen's egg where Marza (1935) found that globulins and pseudoglobulins were detectable in the fully differentiated yolk. The yolk in *Fundulus* contains an appreciable amount of nucleoprotein, much more than the yolk of the hen's egg. Konopacka (1935) has reported the presence of nucleoproteins in the yolk of two fresh-water fishes; she has also identified a mucoprotein in the vesicles of Period 2A.

The presence of nucleoproteins in eggs has stimulated considerable discussion. From an analysis of the ichthulin of various fish eggs Walter (1891), Hammarsten (1905), and Linnert (1909) concluded that there was no nucleic acid present. However, König and Grossfeld (1913) and others have isolated small quantities of purine bases or nucleic acids from the whole eggs of several fishes. The eggs of animals which develop in an aquatic environment contain the constituents of nucleoproteins, while the eggs of terrestrial animals are very poor in them. Needham (1931) has considered these facts and their implications. The histochemical method of Unna (1921) reveals the presence of nucleoprotein in the growing oöcyte of *Fundulus* which confirms the chemical findings in other teleosts. It may be that the positive Feulgen-Rossenbeck (1924) reaction in yolk is conditioned in part by nucleoprotein, but the presence of potassium in the same region confuses the interpretation.

The nucleus of the oöcyte does not give a nucleoprotein reaction at any time (Table IV). Both globulins and albumins occur in the chromatin masses. Nucleoli are rich in acid proteins, but only globulins are present. In the largest nucleoli the globulin reaction at the centers is 2.0 and at the peripheries, 4.0. Smaller nucleoli give a homogeneous reaction like the cortices of the larger ones. The absence of nucleoprotein has been considered in the discussion of the nucleal reaction. Unna's method does not provide data on which a decision can be made concerning the reason for the absence of the nucleal reaction in the nucleus of growing oöcytes.

The Follicular Epithelium and Zona Radiata

It is very striking that the concentrations of the substances recorded in this paper shift conspicuously in the oöcytes during the growth-period. This might be a result of changing resources in the blood stream, or of a selective action of the follicle and zona, or, in some cases, of synthetic activity within the oöcyte in which combination altered the quantity of a substance free to react with the testing reagent.

Since variations in height of epithelial cells have been taken in many cases to indicate degree of metabolic activity, measurements of the width of the follicle were made. At the same time the zona radiata

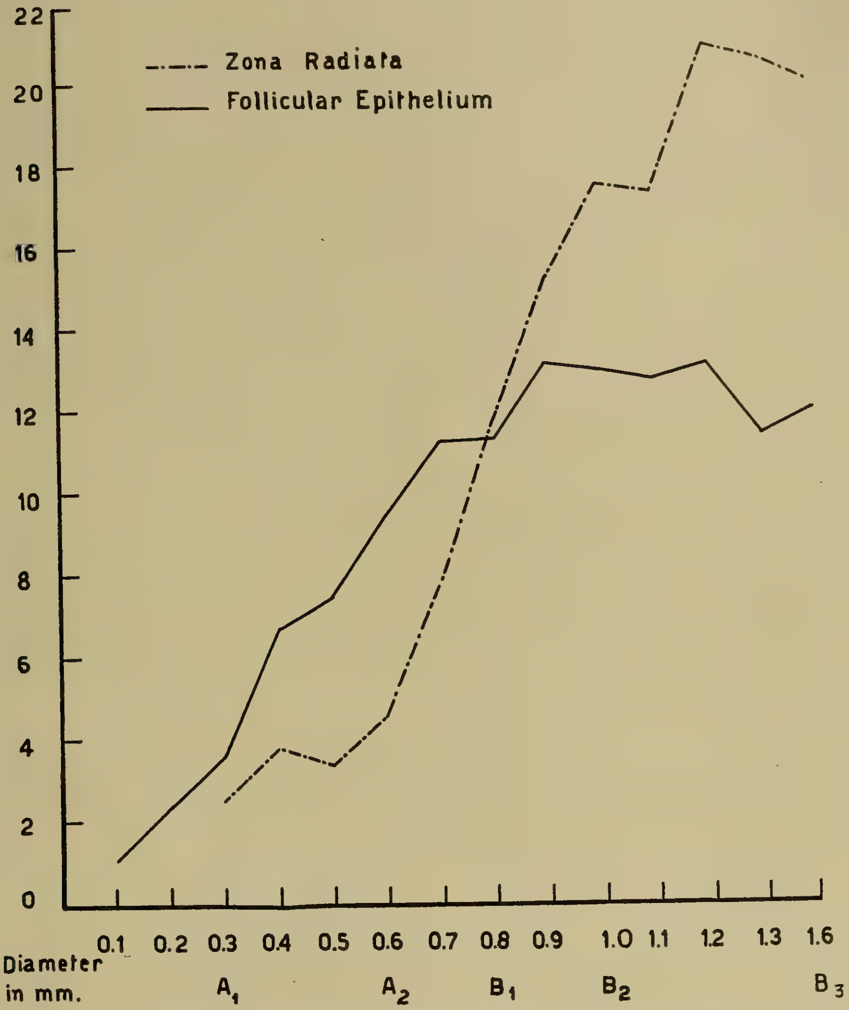


FIG. 19. Variation in height of follicular epithelium and zona radiata of the oöcyte during growth-period. The height in microns is indicated on the vertical axis.

was measured (Fig. 19). The smallest oöcytes, from 10 to 50 microns in diameter, do not have a continuous covering of follicle cells, although some squamous cells may be associated with them (Fig. 1).

When the follicle is complete, in oöcytes from 100 to 150 microns in diameter, its cells do not exceed 1.5 microns in height. During this period the cells of the follicle exercise no inhibitory effect upon the entrance of substances into the oöcyte. The quantity of potassium and acid proteins is relatively very great.

Maximum growth and differentiation of the follicle occurs during Period 2 A, and the height of the cells rises to 11 microns in 2 B_1. The changes in potassium and protein content have been noted. Obviously chemical changes are correlated with the differentiation of the follicle cells. During Period 2 A the large vesicles which are the earliest indication of the unique differentiation of the oöcytes appear, first at the periphery and then throughout the cytosome. One may assume that no longer are the constituents of cytoplasm being delivered in the same relative proportions. Certain substances are accumulating in excess of others. That this is conditioned by the composition of the follicle cells is a simple assumption suggested by the evidence at hand. When the deposition of yolk is occurring rapidly and directly in Period 2 B the follicle undergoes slight change. Some decrease in height is observed as the oöcyte reaches a maximum diameter, and nuclear regression is apparent. The amount of albumins is lowered during this stage.

In contrast to the follicular epithelium, the zona radiata continues to increase in thickness until the end of the growth-period. Some substances can be identified in its canals, but obviously nothing is stored in it. The observations made during this investigation do not suggest that the zona radiata functions in the selection of materials entering the oöcyte.

SUMMARY

The plasmal and nucleal tests and the histochemical methods for detection of iron, potassium, and acid proteins indicate that changes in quality, quantity, and localization of substances characterize the period of differentiation in the oöcyte of *Fundulus*. The oöcyte at the end of its growth-period is morphologically very different from the oögonium. Its structural changes are found to be correlated with the metabolic activities leading to food storage. Cytosomal differentiations are conspicuously chemical phenomena in egg-cells.

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THE DEVELOPMENT OF THE PITUITARY GLAND IN FUNDULUS

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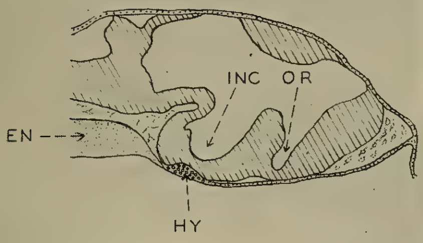
In discussing the structure of the pituitary gland of teleosts Stendell (1914) remarks that in no other group of vertebrates does the hypophysis present such variations, for in nearly every species within this group a different type of structure is encountered. While these can all be reduced to a single generalized plan it is not easy to determine accurately what structures are comparable in the glands of different species. This difficulty is obvious when the glands of *Esox* and of *Fundulus* are compared. According to Stendell, in the pars glandularis of the pituitary gland of *Esox* there is found a small " Hauptlappen " (a term frequently used as a synonym with " pars anterior "; see Tilney, 1936) which is restricted to the anterior dorsal part of the organ, a pars intermedia forming the caudal or ventro-caudal third of the entire gland, and between the two is a region found only in the teleosts and in *Petromyzon,* an " Uebergangsteil." In *Fundulus,* however, (Matthews, 1936) the pars glandularis presents only two distinct regions, a pars intermedia bordering processes of the pars nervosa and forming the posterior pole of the gland, and another part which comprises the major portion of the gland. Does this represent the pars anterior or the Uebergangsteil of other teleosts? A comparison of the cellular structure of this region with that of the pars anterior and the Uebergangsteil of *Esox* is of little value in homologizing these parts, for in *Esox* both the pars anterior and the Uebergangsteil are composed of basophilic cells in general with acidophiles restricted to the neighborhood adjacent to blood vessels, while in *Fundulus* the cells of this major part of the gland are predominantly acidophilic. In its relative size, however, this region in *Fundulus* compares more favorably with the Uebergangsteil, for it is certainly the largest part of the gland (Fig. 5) and Stendell states definitely (p. 92) that the Uebergangsteil is decidedly more voluminous than the principal lobe. Because of this, in describing the structure of the adult gland, I labelled this part the " transitional region," although the appropriateness of the term was questioned. It was thought that a study of the development of the hypophysis in this form might demonstrate which of the parts found in the pituitary gland of other teleosts was lacking in *Fundulus*. Accordingly a series of embryos and young animals ranging in age from two to thirty-eight days after fertilization were fixed, sectioned and studied.

The rudiment of the glandular portion of the hypophysis can first be distinguished five days after fertilization as a small, flattened plate of ectodermal cells lying in close contact with the ventral surface of the forebrain in the region of the future infundibulum (Fig. 1). At this stage of development the foregut is represented by thick cellular masses in which cavities are beginning to appear. Unicellular gland cells described by Armstrong (1936) are already present. In animals six days old this flat plate of ectodermal cells has increased in size, and pushed upward away from the ectoderm so that it now lies slantwise against the posterior ventral angle of the infundibulum which is now quite definite. This epithelial rudiment of the hypophysis is still connected with the ectoderm by a well-defined stalk. These same relationships are present in animals seven days old.

Eight days after fertilization rather pronounced changes in the developing brain can be noted, chief among which is the first appearance of fibrous material in the ventral portion. The epithelial portion of the hypophysis is larger in size and more posterior in position, lying between the posterior wall of the infundibulum and the solid mass of cells which later becomes the foregut and is now heavily marked with the deep-staining unicellular gland cells mentioned above. At nine days of age the epithelial portion of the hypophysis is still further posterior in position (Fig. 2) and the stalk which connects it with the ectodermal region from which it first arose is somewhat longer. The small spaces which formed the first lumen of the gut now form a definite gut cavity but this does not communicate with the outside. At eleven days of age, when the foregut has extended up to the anterior end of the embryo and its lumen is closed off by only a thin membrane, the epithelial portion of the hypophysis lies still more posterior in position although maintaining its relationship to the infundibulum with which it is in close contact dorsally. It is in contact with the entoderm of the gut ventrally and a long, thin strand of ectoderm still connects it with the ectoderm at the anterior end of the embryo. In some cases at this age a few fibers may be found connecting this developing glandular portion of the hypophysis with the infundibulum, although in most cases these fibers appear in later development. Cell boundaries in the developing gland are still indistinct, as they have been throughout the course of development. Between the eleventh and twelfth days of age the strand of ectoderm which has previously connected the epithelial portion of the hypophysis with



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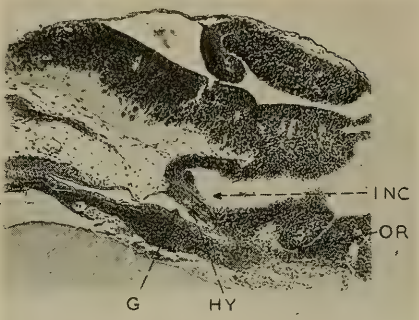

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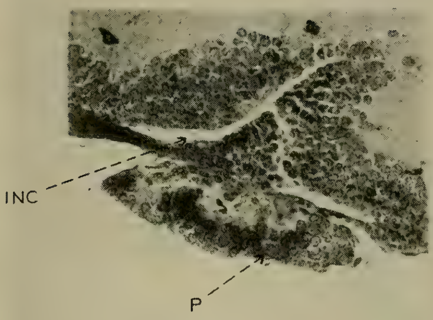

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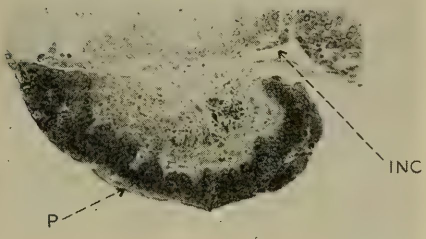

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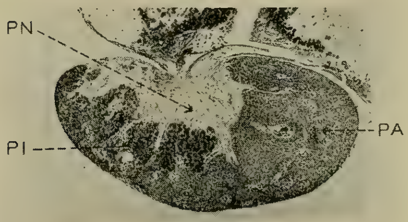

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FIG. 1. *Fundulus* embryo 8 days old, sagittal section of anterior end. Camera lucida outline, × 83. *EN,* entoderm; *HY,* hypophysis; *INC,* infundibular cavity; *OR,* optic recess.

FIG. 2. Embryo nine days old, sagittal section of anterior end, × 85. *HY,* hypophysis; *INC,* infundibular cavity; *OR,* optic recess; *G,* gut.

FIG. 3. Fundulus pituitary gland thirty-two days old, × 315. *INC,* infundibular cavity; *P,* pituitary gland.

FIG. 4. *Fundulus* pituitary gland approximately one year old, × 155. *INC,* infundibular cavity; *P,* pituitary gland.

FIG. 5. *Fundulus* pituitary gland, adult animal, 60 mm. long, sagittal sec, × 50. *INC,* infundibular cavity; *PA,* pars anterior; *PI,* pars intermedia; *PN,* pars nervosa.

the ectoderm at the anterior end of the embryo becomes interrupted and can no longer be traced in embryos twelve days old. The floor of the infundibulum dorsal to the developing pars glandularis has thinned considerably.

From this stage on changes in the developing pituitary gland occur more slowly. By the time the stomodeal membrane has broken through, which occurs in this series between the twelfth and thirteenth days of development, the epithelial part of the hypophysis has attained its adult position in relation to the pharynx and to the brain. It now lies on the ventral surface of the midbrain immediately beneath the ventral wall of the infundibulum, and fibers from the infundibulum join it to the brain. These fibers increase in number until, thirty-two days after fertilization, a short but fairly definite infundibular stalk can be distinguished. These fibers occupy the dorsal border and central portion of the hypophysis, forming the rudiment of the pars nervosa, with the cells that will produce the pars glandularis restricted to the periphery of the organ (Fig. 3). Blood vessels invade the gland between the fifteenth and sixteenth days after fertilization.

During this development of the pituitary gland in *Fundulus* no cavity is to be found at any time. The process in this animal thus differs from that described by Haller (1898), who observed a number of small spaces which appeared in the developing hypophysis of *Salmo,* these spaces eventually coalescing to produce an hypophysial cavity.

The cells of the developing glandular portion of the hypophysis are closely packed, with indistinct cell membranes. Although they do become larger as development proceeds, in the oldest of the animals reared in the laboratory, 38 days of age, the cells are still closely packed around the periphery of the organ and cell membranes are indistinct, with no secretory cells present comparable to those found in the pituitary gland of the adult. The smallest animals in which such cells have so far been found were collected in July, maintained in the laboratory for two months, and measured 26 millimeters when killed in September (Fig. 4). In the pituitary gland of these animals the cells of the pars glandularis are still located around the periphery but they are larger and have definite granules in their cytoplasm. The pars nervosa has also increased in size and processes extend from it into the pars glandularis. Since these animals were collected rather than raised in the laboratory their exact ages are unknown, but in all probability they had been spawned the previous summer and were approximately one year old. Obviously secretory cells first appear sometimes between the sixth week and the twelfth month of development. The exact time of their appearance and their coincidence with other features of development with which beginning activity of the pituitary gland might be concerned must be determined in a series of animals hatched and maintained in the laboratory for longer periods.

Stendell (p. 109) emphasizes the fact that during the development of the pituitary gland of teleosts no cavity is formed. The cells of the solid ectodermal outgrowth proliferate rapidly at its anterior and posterior poles to form the pars anterior and the pars intermedia respectively. As a result of this polar proliferation of cells the region between the anterior and posterior poles, which is less active in development, presents characteristics transitional in nature between the pars anterior and pars intermedia, and constitutes the Uebergangsteil. But here in *Fundulus* apparently not only cells at the anterior and posterior poles but all the peripheral lying cells multiply to produce the pars glandularis. From a comparison of the pituitary gland of year-old animals (Fig. 4) with the developing gland in animals 32 days of age on the one hand (Fig. 3) and with that of adult animals on the other (60 mm. in length, Fig. 5) it is apparent that the main mass of the pars glandularis is produced by multiplication of the peripheral cells found in the early developmental stages. Later those cells adjacent to the pars nervosa may be distinguished from the others, particularly at the posterior pole of the gland where they form a large mass. This is undoubtedly the pars intermedia. The remainder of the pars glandularis is of uniform character. Neither in its development nor in its adult structure can it be called a transitional region between two other parts of the gland. Hence there exists no apparent reason for labelling this major part of the gland an Uebergangsteil. Because of this I now believe that the choice of the term "transitional region" for this major part of the gland of the adult is an unfortunate and confusing one. As a matter of fact, the anterior portion of the gland of *Esox* as described by Stendell differs so much from that part of the gland in *Fundulus* that on the basis of structure alone it is uncertain what parts are comparable in this region of the two glands. Since in *Fundulus* this part in question forms the anterior portion of the organ it may appropriately be called the pars anterior, although it is doubtful whether it is homologous with Stendell's "Hauptlappen."

SUMMARY

The epithelial portion of the pituitary gland of *Fundulus* arises from ectodermal cells that proliferate to form a solid mass which comes to lie between the infundibulum and the pharynx. No lumen occurs in this mass of cells. After fibers from the infundibulum have entered this mass the cells are restricted to the periphery where they multiply to produce the pars glandularis of the adult gland. Those cells adjacent to the pars nervosa can be distinguished from the rest of the glandular portion and constitute the pars intermedia. The remainder of the pars glandularis is of uniform character. No " transitional region " can be distinguished in it either in the developing or in the adult gland. Since this portion forms the anterior part of the organ it may appropriately be called the pars anterior.

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THE SUPPRESSION OF CLEAVAGE IN ASCARIS EGGS BY ULTRACENTRIFUGING[1]

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It has long been known that the division of the cytoplasm of the living egg can be stopped by many external agencies (cold, lipoid solvents, hypertonic and hypotonic solutions, mechanical agitation, etc.) without necessarily inhibiting the division of the nucleus (Wilson, 1902; Chambers, 1924 for references). Furthermore, it is also true that cytoplasmic cleavage may take place in the absence of nuclear material (Harvey, 1936). Accordingly, it is evident that, although nuclear and cytoplasmic divisions are usually correlated they may, nevertheless, be quite independent processes, neither of which is fundamentally dependent upon the other.

In some recent experiments (King and Beams, 1937) on the effects of strong centrifugal force upon the early cleavage stages of *Ascaris* eggs, particularly the diminution process, it was noted that many eggs undergo typical nuclear division in the ultracentrifuge without the accompanying division of the cytoplasm. We are reporting here these experiments together with an analysis of the results in the event that they may have some bearing upon the problems involved in the mechanics of mitosis.

Material and Methods

The eggs used in these experiments were taken from the anterior one-half inch of the uteri of *Ascaris equorum* (= *megalocephala*) variety *univalens*,[2] which had been kept in a refrigerator at 5° C. for not longer than thirty days. Microscopic examination of the eggs taken from this portion of uteri showed them to be in the pronuclear stage of development.

Some of the eggs from each batch were placed in the rotor of the air-driven ultracentrifuge (Beams, Weed, and Pickels, 1933) while in the pronuclear stage, and were centrifuged in 0.9 per cent salt solution at

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[1] Aided by grant from the Rockefeller Foundation for work on Cellular Biology.

[2] It is indeed a pleasure to express here our indebtedness to the authorities of the Hill Packing Company at Topeka, Kansas, for kindly supplying us with the worms.

approximately 150,000 times gravity for varying lengths of time, the maximum being 10 days. In addition, observations were made upon the eggs while centrifuging by means of the centrifuge-microscope recently described by Pickels (1936). In other experiments the eggs were placed under the same conditions in an electric-driven centrifuge, which developed a force of approximately 5,000 times gravity. Part of the eggs from each batch used in an experiment were always kept as controls. All the experiments were done at room temperature which varied from 20° to 25° C.

Sealed cover-glass preparations of the normal and experimental living eggs were studied under the oil immersion lens. It was found that centrifuged eggs placed in 10 per cent formalin solution at 55° C. were coagulated so that the various conditions of stratification are preserved and may be studied at leisure. When permanent slide preparations were made, the eggs were killed in a mixture of 4 parts of 95 per cent alcohol and 1 part of glacial acetic acid and subsequently stained in Heidenhain's hæmatoxylin. In an effort to analyze the components of the various stratified layers a few eggs were killed in Champy's solution at 55° C., and afterwards impregnated in 2 per cent osmic acid for 5 days. By use of antiformin it is possible to remove the shell from living eggs without apparent injury to them.

DESCRIPTION

After sperm entrance and the completion of maturation in *Ascaris,* the eggs shrink and round up giving rise to a rather large perivitelline space which is filled by a watery fluid and enclosed by a very resistant shell (Plate I, Fig. 1). The oöplasm of the egg at this time is heterogeneous in structure, being composed of small brown granules, granular and short rod-like mitochondria (?), clear spherical vacuoles, oil globules and an optically homogeneous cytoplasm. The pronuclei are transparent, rounded bodies located in or near the center of the egg. Surrounding the egg is a delicate plasma membrane, the morphology of which is not discernible even under the oil immersion lens.

Upon centrifuging at 150,000 times gravity the eggs are stratified almost immediately into the following layers: (1) oil globules at the centripetal pole, (2) a zone of clear vacuoles, (3) transparent cytoplasm, and (4) brown granules at the centrifugal pole (Plate I, Fig. 2). The nuclei and also the mitotic spindle in dividing eggs are included in the clear cytoplasmic layer. The mitotic spindle is oriented at right angles to the centrifuge axis and parallel to the stratified layers. Unlike the condition in embryonic chick cells, the elements of the mitotic spindle are not distorted. This indicates that their relative specific gravity is approximately the same as that of the cytoplasmic layer. When viewed through the microscope-centrifuge, the eggs were observed to be somewhat elongated along the centrifuge axis, so that the centrifugal and centripetal ends of the eggs are in direct contact with the shell, which no doubt aids in preventing their fragmentation. Eggs in the pronuclear stage may, in rare cases, be divided into three parts within the shell as follows: fat at the centripetal pole, protoplasm and clear vacuoles in the middle and brown granules at the centrifugal pole (Plate I, Fig. 3). In other more frequent cases only the oil globules are completely rounded off from the rest of the egg at the centripetal pole, and flattened in the form of a disc (Plate I, Figs. 6, 7, 8, 9, 11). Eggs of this kind, if removed from the centrifuge before cleaving, frequently develop into normal larvæ, with the flattened disc of oil globules within the perivitelline space. However, if the eggs are removed from the centrifuge before the oil globules are completely cut off they become reincorporated into the egg through a narrow stalk, and eventually are redistributed as are the other layers of the egg. In Plate I, Fig. 4, is illustrated a centrifuged egg with conjugating pronuclei that had been killed in Champy's solution and subsequently impregnated in 2 per cent osmic acid. It will be noted that a dark layer, which is thought to contain the mitochondria, appears just centripetal to the brown granules. This layer of small granules can also be seen in the living egg under suitable conditions. It had previously been thought that the brown granules represent the mitochondria in *Ascaris* eggs (Fauré-Fremiet, 1913). The dark granular layer just centrifugal to the fat globules may possibly represent the Golgi material.

If the eggs are left in the centrifuge until the controls have undergone cleavage a typical mitotic apparatus (centrosomes, asters, spindle and chromosomes) is formed (Plate I, Fig. 5), giving rise to two nuclei within the cell because of the failure of the cytoplasm to cleave. (The cytology of the mitotic figure and the chromosome behavior in such eggs is to be reported elsewhere—King and Beams, 1938.) These two nuclei, in turn, may form typical division figures simultaneously (Plate I, Fig. 6), giving rise to four nuclei within the uncleaved egg (Plate I, Fig. 8); the four nuclei may divide in the same manner (Plate I, Fig. 11), each division corresponding to a cleavage in the controls. In fact, the first two nuclear divisions often take place at about the same rate as the first two cleavages of the controls. However, the subsequent cleavages often become more or less irregular and out of step with the controls.

If the eggs are removed from the centrifuge after one, two or more divisions of the nuclear material only, a curious surface phenomenon is noted in the region of the clear cytoplasmic layer. Immediate examination of the eggs upon removal from the centrifuge often reveals many small and a few quite large pseudopodium-like processes flowing out of the egg; these are in many cases completely cut off (Plate I, Fig. 7). This material which has been stratified is thought to be or to give rise to the " surface active material " which breaks out and precipitates, forming a film or plasma membrane. The presence of such a material in the egg is assumed to be necessary in the regions where the cleavage fur-

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PLATE I

Explanation of Figures

All the figures were drawn from living eggs by Mary Crosten. The amount of the centrifugal force has been in all cases 150,000 times gravity unless otherwise indicated. The direction of the centrifugal force in all cases except Fig. 1 was approximately toward the bottom of the plate.

Fig. 1. Pronuclear stage of a normal *Ascaris* egg showing the general structure of the cytoplasm and the position of the pronuclei.

Fig. 2. Egg as in Fig. 1 after centrifuging for one-half hour. Materials stratified with fat at the centripetal pole, then a layer of clear vacuoles, next a layer of clear cytoplasm containing the pronuclei, and finally, a layer of brown granules at the centrifugal pole.

Fig. 3. Unusual condition of egg fragmented into three parts: fat and clear vacuoles in the centripetal fragment, cytoplasm and pronuclei in the middle fragment and the brown granules in the centrifugal fragment. Centrifuged two hours.

Fig. 4. Egg fixed in Champy's solution immediately upon removal from the centrifuge, and subsequently impregnated in 2 per cent osmic acid. Stratification clearly shown. The dark layer between the nucleus and the brown granules is thought to represent the mitochondria. The darkly impregnated layer just centrifugal to the fat globules may possibly represent the Golgi material.

Fig. 5. Egg undergoing division of the nucleus without division of the cytoplasm. The asters are faintly shown.

Fig. 6. Prophase stage of the two nuclei which correspond to those in the second cleavage of the controls. Small masses of " surface active material " shown budded off from the egg.

Fig. 7. Drawing of egg removed from centrifuge after the controls had undergone one division. The formation of the pseudopodia in the region of the cytoplasmic layer is shown.

Figs. 8–9. Eggs that have undergone two mitotic divisions of the nuclear material without division of the cytoplasm in the centrifuge. Large fragments are shown which were actively undergoing ameboid movement. The oil globules are shown fragmented from the egg at the centripetal pole. Drawings made after a partial redistribution of the stratified layers had taken place.

Fig. 10. Egg removed from centrifuge at the time the controls had undergone the first cleavage. Many pseudopodium-like processes shown at the periphery of the egg, none of which in this particular case was fragmented off. Drawing made after partial redistribution of the stratified layers.

Fig. 11. Egg that had passed through three mitotic divisions of the nuclear material in the centrifuge. One nucleus is seen cleaved off into a small cell of clear cytoplasm. A redistribution of the brown granules in the egg has taken place. Fat globules are shown at the centripetal pole.

Fig. 12. " Ball " egg that divided in centrifuge at 5,000 times gravity. Most of the brown granules are cleaved off during the first division. The stratification of the various materials is clearly shown.

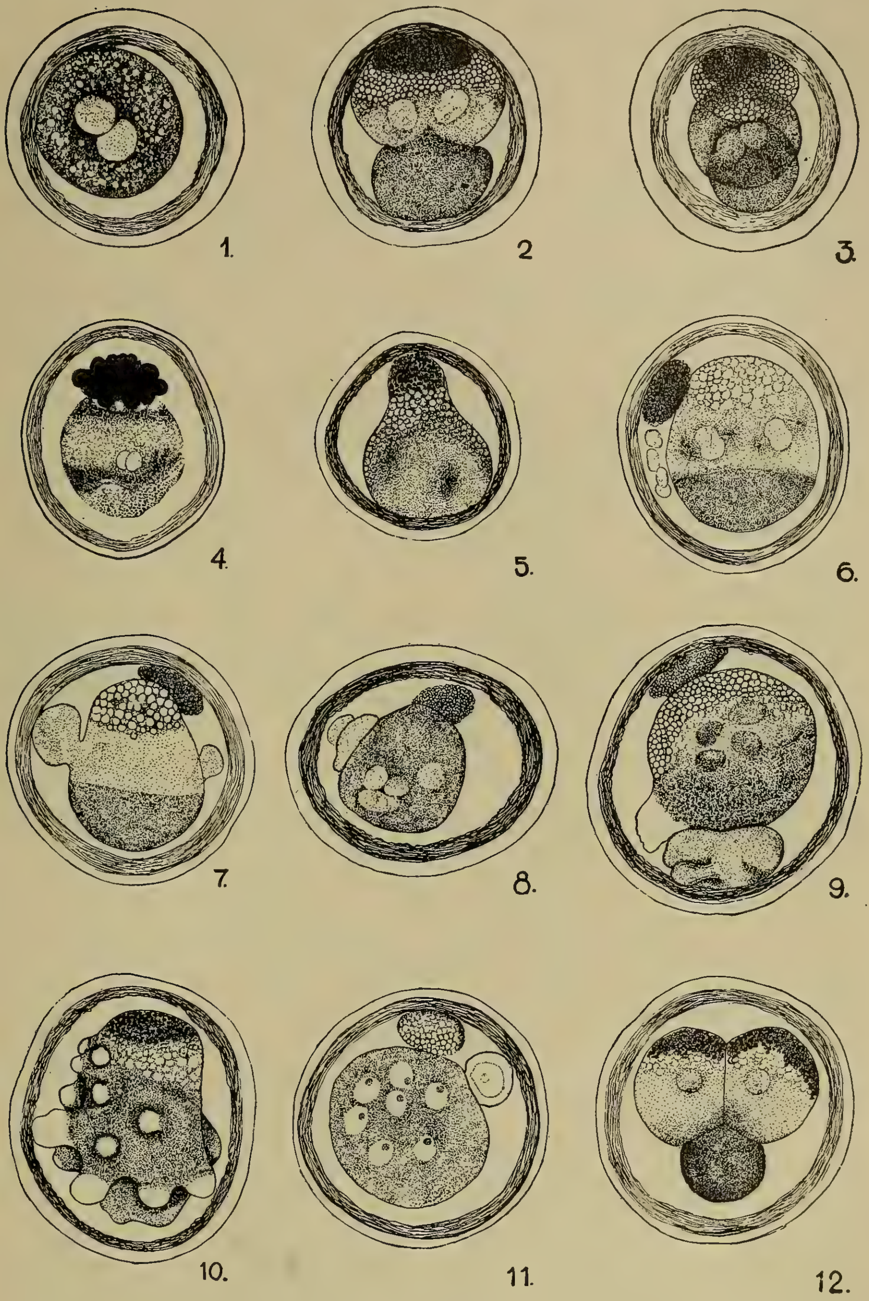
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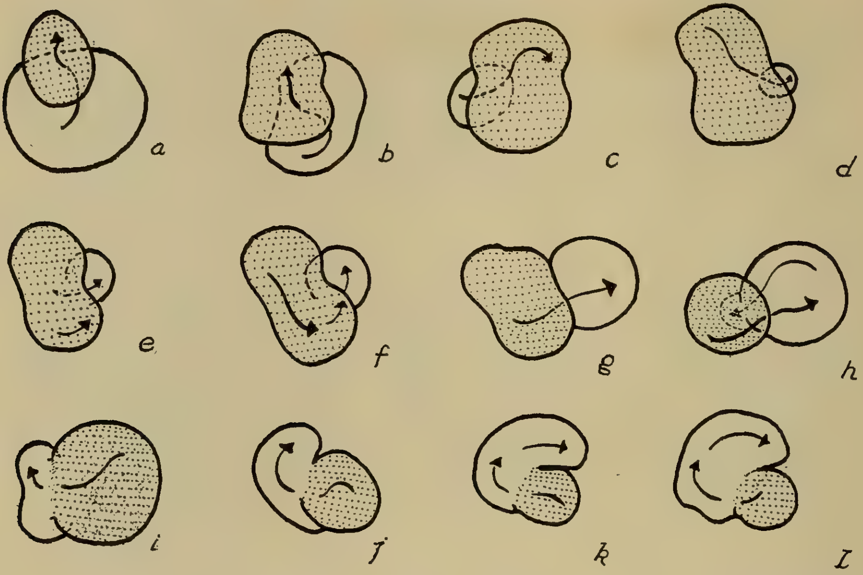
PLATE I

rows are destined to form. The fragments show very striking and rapid ameboid movement within the perivitelline space. In text fig. 1 (*a–l*) are illustrated diagrammatically the consecutive movements of a typical fragment like that shown in Fig. 8 during an interval of 5 minutes. Such active movement has been noted to continue for four or five hours, after which time the fragments may divide into many smaller ones. We have not observed that these bodies subsequently fuse with the egg.

In other less frequent cases, where the eggs were treated in a similar way, many small pseudopodium-like processes are formed at the surface of the clear and granular layers but they do not become separated off (Plate I, Fig. 10). Such pseudopodia upon division of the egg outside of the centrifuge are reincorporated with the blastomeres. It is an interesting fact that frequently one observes eggs (such as those shown in Plate I, Fig. 11), where one or more small cells with clear cytoplasm have been cleaved off in the centrifuge. This condition probably occurs when one of the mitotic spindles is sufficiently close to the periphery of the egg in the presence of " surface active material."



Text Fig. 1, *a–l,* diagrams showing the ameboid movement of a typical fragment like that shown in Plate I, Fig. 8, during an interval of 5 minutes.

Those eggs that have undergone one or two divisions of the nuclei without division of the cytoplasm in the centrifuge will, upon removal from the centrifuge, frequently divide into twice as many cells as there were nuclei present. This division, however, takes place only after partial or complete redistribution of the stratified materials.

Ascaris eggs like those of the sea-urchin (Harvey, 1933) will divide in a gravitational field of 5,000 times gravity. In most of the eggs the cleavage is complete. However, in a few it is incomplete, as it does not pass through, but around the brown granular layer at the centrifugal pole, giving rise to what Boveri (1910) and Hogue (1910) have called "ball" eggs (Plate I, Fig. 12). The fact that these eggs undergo cytoplasmic cleavage may be explained by assuming that the force used, although sufficient to produce stratification of the visible materials, has not been great enough to displace and stratify the "surface active material." This assumption is supported by the fact that eggs centrifuged at 5,000 times gravity while dividing do not usually form pseudopodia as do those centrifuged at higher speeds.

No attempt has been made here to determine the absolute viscosity of the protoplasm of *Ascaris* eggs. However, the relative viscosity has been determined at room temperature by the centrifuge method, and by observations upon Brownian movement in the eggs at various stages in the mitotic cycle. Fauré-Fremiet (1913) has also used the centrifuge method to study the viscosity of *Ascaris* eggs at different temperatures. In our experiments the eggs, which were in different stages of division, were placed in the electric machine and centrifuged at 5,000 times gravity for 20 minutes. The relative position or layering out of the brown granules was used as an index of the relative viscosity of the eggs, in the pronuclear stage, prophase, metaphase, anaphase and telophase. Brownian movement was followed in eggs that were undergoing division, as was the redistribution rate of the brown granules after they had been displaced by the centrifuge.

The evidence from these observations indicates that the egg is relatively fluid during the pronuclear and prophase stages. During metaphase the egg becomes progressively more rigid and Brownian movement usually ceases except perhaps at the periphery. During anaphase and telophase the eggs show the highest degree of viscosity. At the end of telophase when the asters begin to recede, and the reorganization of the nuclei takes place, the viscosity gradually becomes low again.

It should be pointed out that, during the prophase, when the amphiaster is forming, a gelation or relatively high viscosity is present in the region of the immature or small aster. It seems probable that the viscosity is as high in this region as it will ever be, and that the following stages, which show a higher viscosity for the egg as a whole, are due primarily to a spreading of this gelation from the region of the small aster toward the periphery of the egg, a condition associated with the development and growth of the asters. (For a recent review of the literature on viscosity studies see Fry and Parks, 1934.)

Discussion

Cell division under normal conditions appears to be accomplished by a series of complex integrated phenomena, involving both the nucleus and cytoplasm; this becomes manifest, in part at least, through changes in viscosity, appearance of the mitotic figure, and cleavage furrow. These morphological changes in the dividing egg are, no doubt, the expression of what might be termed a " mitotic field of force," by which a part of the work necessary for cell division is accomplished. It is, however, beyond the scope of this paper to discuss the whole mechanism of cell division, but a few remarks upon the process of cytokinesis in the light of our results seem desirable.

It should be pointed out at the beginning that the failure of the *Ascaris* eggs to cleave in the ultracentrifuge can not be attributed to any marked change in the morphology or position of the mitotic figure, as its form is exactly comparable to that of the normal egg, and its position in the cytoplasmic layer is the same as that in eggs which undergo cleavage in the centrifuge at 5,000 times gravity. Furthermore, contrary to the views expressed by some workers (Chambers, 1924; Harris, 1935), no evidence was observed which would indicate that the strong centrifugal force used in our experiments did in any way affect the normal viscosity cycle during nuclear division. In this respect our results differ from those of most other investigators who have inhibited cell division by external agencies (cold, ether, hypertonic and hypotonic solutions, mechanical agitation, etc.), in that these methods affect the viscosity of the cell, resulting in a partial or complete disintegration of the asters, which, in turn, inhibits cell division. In other words, " cells which possess asters under normal conditions absolutely depend upon the presence of asters for cytoplasmic division " (Chambers, 1924). It is evident, therefore, that the inhibition of cytoplasmic cleavage in the *Ascaris* egg by the ultracentrifuge can not be explained upon the same basis as the cases cited above.

It is a well established fact (Erlanger, 1897; Conklin, 1917; Speck, 1918; Strangeways, 1922; Chambers, 1924; Gray, 1931; Harris, 1935, and others) that in many dividing cells, particularly the nematode egg, there are important peripheral changes which take place in the cytoplasm, which seem to be associated with the development of the amphiaster, and which have a distinct bearing upon cell division (see Speck, 1918 and Chambers, 1924 for literature). In general, it may be said from the results of the above-named investigators, that when the asters develop to the point of almost reaching the periphery of the egg, a decrease in peripheral viscosity becomes noticeable. In this relatively fluid periphery it is possible to see a flow, initiated by a change in surface tension, which, according to all the investigators who have described it, is directed toward the equatorial region of the cell. It is not possible in the *Ascaris* egg to observe a flowing movement in the peripheral region during division, but it is significant that here, particularly at the equator, cessation of Brownian movement is generally last observed during metaphase. Furthermore, in a few instances, it was found that, during the normal mitosis of *Ascaris* eggs, a distinct " bubbling " or protrusion of a relatively clear material in the form of small pseudopodia was observed, especially at the equatorial region. Kautzsch (1912), and Painter (1915) have likewise described marked conditions of this kind in *Ascaris* eggs in which, apparently, the normal division had been delayed. This activity or mobility at the surface of dividing cells has frequently been observed, particularly in tissue culture cells (Strangeways, 1922).

It seems not unreasonable to suppose, therefore, that the relatively fluid peripheral material is formed during the gelation of the amphiaster, or at the solution of the nuclear membrane, and then is forced to take up a position at the periphery of the egg, where it seems to function in the formation of the cleavage furrow.

The fact that the marked formation of extraovates takes place in the region of the cytoplasmic layer of eggs, that have undergone one or more typical nuclear divisions in the centrifuge although cleavage has been suppressed, may be explained by the assumption that the peripheral surface active, membrane-forming material is stratified in this region. Our observations indicate that at each division of the nucleus more of the " surface active material " is formed, and, if in the centrifuge, is stratified so that when the centrifugal force is released, some of this material, which changes surface tension, is extruded, and frequently is completely separated from the egg. Ameboid activity ensues until all the surface energy is dissipated. If this interpretation is correct, and, if the surface active material is essential for film or membrane formation, as there is good evidence for believing (Heilbrunn, 1928), then one need only assume that the inhibition of cleavage of the *Ascaris* eggs in our experiments is due to a concentration of the material into a layer parallel to the axis of the mitotic spindle, thus depleting the egg of the membrane-forming material in the regions where the cleavage furrows would normally form. This interpretation, we believe, is in harmony with the highly significant work of Heilbrunn (1928) on the " surface active material " of *Arbacia* eggs. He found, after stratifying of the visible materials in the eggs by means of the centrifuge, and subsequent crushing, that a surface film was formed only at the centrifugal pole, the position taken up by the pigment granules. Heilbrunn, therefore, concluded that the " surface active material " had been displaced in the centrifuge, and that it was the pigment granules or material stratified in the same region which is or gives rise to the " surface active material."

There are, no doubt, some who will interpret the failure of the cytoplasm to cleave in the centrifuge as simply mechanical, because of the intense stratification or " packing " of the elements in the stratified layers. This condition of stratification may possibly be a factor; but it has been shown by Boveri (1910), Hogue (1910), and in this paper, that cleavage will take place in the centrifuge when the visible materials are clearly stratified. It is only when the centrifugal force has been very great that division fails to occur. Why the cytoplasm fails to cleave in the region of the dividing mitotic figure is not known, but one might assume that the " surface active material " is stratified into a layer which does not include the mitotic spindle; or perhaps because of its stratification, the " surface active material " has become temporarily inactivated.

It should be pointed out that in certain biological material, the formation of a plasmodium or syncytium is the normal condition. For example, in the developing insect egg, the first divisions involve the nucleus only; the cleavage of the cytoplasm takes place later after the nuclei have migrated to the surface of the egg. No satisfactory explanation for this condition has ever been suggested, but might it not be because of the lack of a sufficient quantity of some material essential for cytoplasmic cleavage, such as, for instance, the surface active, membrane-forming material? Or could it be that the materials essential for cytoplasmic cleavage can not function properly in the interior of the egg?

In a preliminary report (Beams and King, 1936), it was shown that eggs of *Ascaris suum* were not killed by centrifuging at 400,000 times gravity for 30 minutes; the same condition holds true for eggs of *Ascaris equorum*. These facts are interesting in the light of the recent work of Svedberg (1934) on strongly centrifuging non-living colloidal systems. He found that certain of the large molecules may be thrown out of solution at 75,000 to 200,000 times gravity. Tobacco virus proteins may be likewise thrown out of solution and crystallized at 200,000 times gravity (Stanley and Wyckoff, 1937), and recently studies have indicated that it might be possible to separate isotopes by the ultracentrifuge method (Beams and Haynes, 1936). One might assume, therefore, from these facts, that a similar separation of the light and heavy molecules might take place in protoplasm at such strong forces. In fact, Moore (1935) has recently concluded that the living elements of a plasmodium consist of heavy and light components, which, when centrifuged at 75,000 times gravity for 5 minutes, are separated, and proliferation stopped. However, if allowed to rest for sufficient time, the separated elements return to their normal spatial relationship and proliferation is resumed. Our work shows definitely that *Ascaris* eggs will live and undergo nuclear division in the centrifuge at 150,000 times gravity for at least ten days. Because of this fact we do not believe that any marked separation of protoplasmic structure in the manner suggested by Moore has taken place. On the other hand, it seems more likely that forces, perhaps electro-static in nature, are sufficiently great in *Ascaris* protoplasm to prevent a disruption of its architecture by such strong centrifugal forces, or, less likely, perhaps the living units of the protoplasm may all be of the same specific gravity.

CONCLUSIONS

1. Eggs centrifuged at 5,000 times gravity are stratified into the following layers: (1) fat at the centripetal pole; (2) clear vacuoles; (3) clear cytoplasm containing the pronuclei or the mitotic spindle; the latter usually oriented with its long axis at right angles to the centrifugal force and parallel to the layers; and (4) brown granules at the centrifugal pole. When centrifuged at 150,000 or 400,000 times gravity and subsequently fixed in osmic acid solution, two additional layers are noted: one just centripetal to the brown granules, the other just centrifugal to the fat layer; the former is believed to represent the mitochondria; the other may possibly represent the Golgi material. At these high speeds the brown granules rarely become fragmented from the egg. However, the oil globules are frequently cut off at the centripetal pole.

2. Eggs in the pronuclear or early prophase stages will, upon being placed at 5,000 times gravity, undergo the early cleavages at about the same rate as controls. In a low percentage of eggs the brown granules are cut off at the first cleavage, which usually takes place at right angles to the stratified layers.

3. Eggs in the pronuclear or early prophase stages that are centrifuged at 150,000 times gravity undergo typical mitotic division of the nucleus without the usual division of the cytoplasm. This gives rise to two nuclei within the egg, which may in turn form typical mitotic

figures and divide at the same time, giving rise to four nuclei within the uncleaved egg. The first two or three divisions of the nuclear material often correspond in tempo to the first two or three cleavages of the controls.

4. Eggs that have undergone one or more divisions of the nucleus without the division of the cytoplasm show, upon immediate examination after removal from the centrifuge, a curious peripheral " bubbling," or the extrusion of pseudopodium-like processes in the region of the clear cytoplasmic layer. These pseudopodia may be cut off from the egg, in which case they undergo very rapid ameboid movement. If not detached they are withdrawn into the egg during the redistribution of the stratified materials and subsequent cleavage.

5. The material, which is extruded in the form of pseudopodia, is thought to represent " surface active material "; a material which upon coming in contact with the surface or outside environment of the egg coagulates, giving rise to a film or plasma membrane. It is this same material which during normal cleavage concentrates at the periphery of the egg and functions in the formation of the cleavage furrow.

It seems probable that the increase or growth of the " surface active material " is not markedly inhibited by high speed centrifuging, so that at each division of the nucleus more of it is formed, and becomes stratified. Thus, upon cessation of the centrifuging, part of this material is extruded, a membrane is formed upon coming in contact with a foreign environment and active ameboid movement is initiated.

6. Uncleaved eggs centrifuged at 400,000 times gravity for 30 minutes are not killed, and, if the stratified materials are redistributed before cleavage takes place, they apparently develop normally.

7. No evidence was found which indicates a separation of the ultramicroscopic cytoplasmic components had taken place.

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A STUDY OF THE BACTERIAL AND ALLEGED MITOCHONDRIAL CONTENT OF THE CELLS OF THE CLOVER NODULE

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INTRODUCTION

In a paper recently published the author expressed the view that certain granular and rod-like cytoplasmic inclusions occurring in *Amœba proteus* and *Arcella vulgaris* belonged in the category of bacteria, probably symbiotic or commensal, while still other similar inclusions appeared to be only temporary bacterial invaders of the cytoplasm. Certain of these granular and rod-like inclusions had, in the past, been described as mitochondria. In the course of this investigation the initial intention was to employ the clover nodule as material for making a comparative study between certain cell inclusions, alleged mitochondria, as they appeared in the nodule, and similar inclusions occurring in *Amœba* and *Arcella.* From available literature the clover nodule promised to be very valuable material for making such a comparative study. Certain applied tests, however, induced me to discard that material for the time, with the hope of being able later to publish my findings separately.

The phenomenon involving bacteria and alleged mitochondria as it occurs in the nodule of the clover is quite puzzling. The problem of making a clear distinction between the two by present-day methods of technique has previously been recognized by investigators, including Buchner (1921).

Techniques, similar to that employed in arriving at the above-mentioned conclusions regarding *Amœba* and *Arcella,* have likewise been employed here in an intensive study of the contents of the clover nodule. Since this material can easily be secured throughout the year and since the nodules contain bacterial forms, including both rods and granules, varying in size from minute forms which approach the limit of microscopic vision to forms of relatively large size, it becomes quite evident that such material offers an excellent opportunity for the study of symbiotic relationship, as well as for making a comparative study of different cell constituents.

Mitochondria have been described in the nodule of clover in association with the different pleomorphic forms of recognized bacteria. Cowdry (1923) has presented characteristics which he considers serve to distinguish between small bacterial forms and mitochondria, as they occur in the nodule of the white clover. Differentiation between bacteria and alleged mitochondria has been made largely on the grounds of staining reactions and responses to well-known lipoid solvents. It is the purpose of this investigation to show that size, staining ability, reaction to Janus green, and lipoid solvents are not sufficient to distinguish bacterial forms and apparent mitochondria as they occur in the clover nodule.

From the following observations upon many nodules, treated with different methods of technique, smears, cultures on artificial media of bacteria from both the nodule and from the soil, I am convinced that an important bacterial type, or possibly types, as well as their general staining qualities, have been overlooked.

Wallin (1922) is of the opinion that a minute type of bacteria, not unlike mitochondria in appearance, exists in the nodule of the white clover. He designates them " juvenile forms " and believes them to be young bacilli which have recently entered the nodule from the soil. He asks (p. 458) : " What evidence may be submitted to indicate that these bodies, generally considered cytoplasmic organs, are not bacteria that have gained entrance to the plant through the root-hairs? The author has not been able to find any evidence that would satisfactorily answer these questions."

In the present paper, it is my aim to deal chiefly with the minute rod-like and granular inclusions occurring in the clover nodule and in the soil and to submit evidence which further indicates that minute forms of bacteria have been confused with mitochondria.

Material and Methods

Clover nodules were secured from various locations, during different seasons of the year, and from soils varying in moisture content. While the nodule of the white clover was chiefly used during this investigation, nodules of crimson clover were likewise studied. Mitochondrial fixatives, including Altmann's, Regaud's, Champy-Kull's, Flemming's, and Murray's, were employed. In addition to mitochondrial fixatives, Schaudinn's fluid with iron-hematoxylin was used. In some cases, prior to fixation, nodules were treated with 95 per cent alcohol or ether for 12 to 24 hours. Still others were fixed in Altmann's fluid to which had been added 5 per cent of acetic acid. In order to avoid as much as possible variations which naturally occur in structure and content of different nodules, as well as in different regions of the same nodule, nodules were severed longitudinally in nearly equal halves and the halves subjected to different treatment. The two halves of the same nodule were then placed side by side, embedded, sectioned, stained and mounted.

Nodules were always thoroughly washed in sterile water before cutting. After cutting on a slide, in a drop of sterile water, smears were made of that portion of the contents normally lost during the cutting procedure. The smears, likewise, served in making comparative studies. In addition to treating the nodule as a whole, smears of entire nodules were made. All smear preparations were allowed to dry completely before further treatment. While some were treated with alcohol or ether before applying mitochondrial fixatives, others were not.

Supravital tests were made on the contents of the clover nodule by thoroughly crushing the nodule upon a slide which had previously been filmed with a 1–20 alcoholic solution of Janus green (Grübler's) prepared from a 1 per cent stock solution in absolute alcohol. Comparative tests of the nodular contents were made by examining similarly treated nodules which were not exposed to Janus green. For permanent staining, anilin-fuchsin with methyl green or toluidin blue counterstain and iron-hematoxylin were employed.

In the following illustrations granules and rods which color red in the stained material are drawn in black, while those staining blue, green, or indefinitely are illustrated in diluted ink.

Artificial media were inoculated with contents of the nodule, as well as with soil which had been removed from the roots. Two different kinds of media were used for this purpose: a mannite medium, free from combined nitrogen and containing a fermentable carbohydrate, the formula for which will be found in Topley and Wilson (1932), p. 315, Vol. 1; for plating a 2 per cent agar plus 1 per cent lithium chloride.

I wish to make grateful acknowledgment to Dr. H. E. Jordan of the Department of Histology and Embryology, who placed at my disposal the facilities of his laboratory, for his very valuable aid and kindly criticism during the course of this investigation and preparation of this paper.

OBSERVATIONS

Wallin describes and illustrates three distinct types of bacteria-like organisms as they occur in the nodule of the white clover. One of these types, consisting of minute rods and granules, he found located in the distal or younger portion of the nodule. He states: "In Fig. 3 the cells contain small bodies that are not unlike mitochondria in appearance.

These forms, undoubtedly, are the young bacilli that have recently entered the nodule, or they represent a young form that will later metamorphose into the mature type. They may be designated the 'juvenile forms.'" Cowdry, on the other hand, thinks: "that Wallin's questions may be answered through the simultaneous and differential demonstration of mitochondria and Bacillus radicicola side by side within the same cells." In other words, Cowdry is of the opinion that mitochondria and bacteria can be distinguished in the nodule on the grounds of morphology, differential staining, arrangement in the cell, fixing with Bouin's fluid and 95 per cent alcohol. Cowdry also states: "In figures 1 and 2 only mitochondria are shown. They occur in about the same number in neighboring cells and are, for the most part, rod-like, and occasionally filamentous, not spherical, as indicated by Wallin in his figures 6 and 7."

Among the many nodules examined during the present investigation, much variation in nodular contents has been encountered. In matter of fact so much variation occurs with respect to cellular structure, nature, and arrangement of cellular contents, morphological bacterial types, rods and granules, differential staining, that one is at a loss to determine just what constitutes the rule. Nodules occur which have little cellular arrangement, the cytoplasm being in a continuous, unlimited mass, in so far as the cell walls are concerned. Minute rods and granules sometimes occur in this cytoplasmic mass in abundance. In some sections the large majority stain red and have the appearance of mitochondria. In later sections of the same nodule apparently the same inclusions sometimes show more differential staining qualities, a lesser number staining red while more are stained green, until finally very few, if any, present a mitochondrial appearance, but stain green. Other nodules present relatively large cavities devoid of cytoplasm, or cells with little or no cytoplasm, and these may be partially or completely filled with bacteria-like inclusions. These inclusions may vary slightly in size in some cases, but many are very minute (Plate I, Figs. 1 and 2). They frequently stain red, while in some cases they show differential staining properties.

Minute rods and granules which color red occur in some cells which possess large metamorphic forms of *Bacillus radicicola* and are distributed peripherally, as Cowdry has shown, or they may be scattered more or less uniformly throughout the cell (Plate I, Figs. 3 and 4). Among these, one finds a certain amount of differential staining in some cases. I have been unable to find any evidence that strict peripheral arrangement of these minute inclusions in such cells is the rule, as Cowdry has emphasized. They appear as rods, short filaments, or granules.

Seldom does one find two nodules, even from the same plant-root, presenting the same picture with regard to bacterial or minute bacteria-like content. Different metamorphic forms of *B. radicicola* appear in the same nodule, but from the fact that I have never observed the entire metamorphic series within a single nodule, I conclude that a complete series within a single nodule is most probably an exception and not the rule. In fact, in many nodules, one single form seems to predominate throughout. Usually, there are several forms present.

Rods and granules, many of which are minute and approach the limit of clear microscopic vision, are found within some of the nodules. In some cells they are abundant, while in others they are sparsely scattered (Plate I, Figs. 5 and 6). In some nodules, apparently, they are absent, while in others only a small percentage of the cells possess them. Their staining ability varies, even within the same cell; i.e., some will stain red, while others stain green or blue (Plate II, Figs. 7 and 8).

The fact is well recognized that properties such as the above, alone, are not sufficient to determine the true nature of small cytoplasmic inclusions, making it necessary to seek additional evidence. One of the recognized criteria for demonstrating mitochondria after mitochondrial fixation is their ability to color red with anilin-fuchsin, followed by a counter-stain of methyl green or toluidin blue. A significant fact here is that, among the numerous nodules investigated during the present

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EXPLANATION OF PLATES

All drawings were made with the aid of a Bausch and Lomb 1.8 mm. oil-immersion objective and a 10 × ocular. All figures with the exception of Fig. 18 (Plate III) were taken from preparations treated with mitochondrial fixatives and stained with anilin-fuchsin and counterstained with methyl green or toluidin blue. Figure 18 was taken from a preparation treated with Schaudinn's fluid and stained in iron-hematoxylin. Magnification about 1,200.

Plate I

Explanations of Figures

Fig. 1. Section of a nodule with the lumen partially filled with bacteria-like rods which show some differential staining. Altmann's fixation.

Fig. 2. Section of a nodule with cells and lumen containing both rods and granules. Shows some differential staining. Regaud's fixation.

Fig. 3. Cells from a nodule, showing mature forms of *B. radicicola* together with numerous minute granules and rods located in different regions of the cell. Shows some differential staining. Regaud's fixation.

Fig. 4. Two cells from a nodule, showing minute granules and rods scattered among the mature bacterial forms. Murray's fixation.

Fig. 5. Cell from a nodule, showing minute cell inclusions. Flemming's fixation.

Fig. 6. Section of a nodule, the cells of which contain many rods and granules. The greater number stain red, a few green, while some are indefinitely stained. Champy-Kull's fixation.

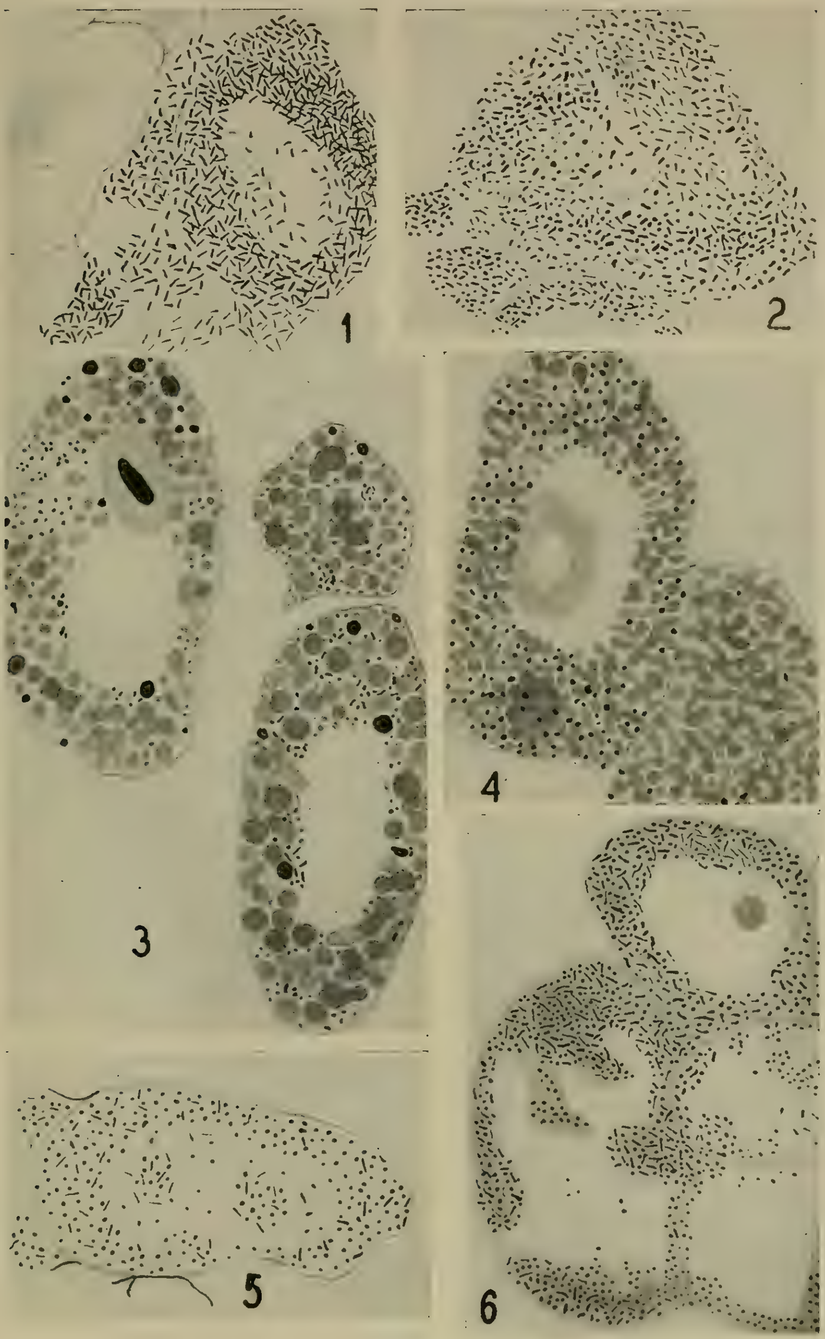

PLATE I

work, a relatively small percentage of the cells examined presented granules or rods suggestive of mitochondria. More rarely, in comparison, have there been cases of cells possessing the larger metamorphic bacterial forms and at the same time exhibiting minute rods or granules, or both, suggesting mitochondria. In some cases where this latter condition has prevailed the minute forms have shown differential staining properties (Plate I, Fig. 3 and Plate II, Fig. 8).

Nodules which had been treated with 95 per cent alcohol or ether for 12 to 24 hours before fixation, or with Altmann's plus 5 per cent of acetic acid, have shown minute granules and rods, sometimes in relatively large numbers. Many of these inclusions stain red with anilin-fuchsin and toluidin blue. Like those appearing in nodules which had not been subjected to the above-mentioned treatment before fixation, they vary to some extent in size, sometimes in the same cell. Figures 9, 10 and 11 (Plate II) illustrate results obtained in different nodules which had been treated in 95 per cent alcohol for 12 to 16 hours and fixed in Murray's fluid and stained with anilin-fuchsin and toluidin blue. Granules and rods can be observed scattered more or less uniformly throughout the cell. While some of these inclusions are stained faintly red and others are almost completely overshadowed by numerous large bacteria, still others can be observed much more readily.

In Fig. 12 (Plate II) a cell containing granules and rods stained red can be observed and illustrates one of many cases following treatment with 95 per cent alcohol for 12 hours and fixed in Altmann's fluid. It will be noted that the cell is completely filled with minute bacteria-like forms and that there is a close likeness between the forms that stain red and those that stain green. Figure 14 (Plate II), and Fig. 15 (Plate III) are drawings made from two separate nodules which were treated with ether for 12 hours before fixing with Champy-Kull's fluid and stained in anilin-fuchsin and methyl green. These two specimens show much variation in number, form and arrangement of granules and rods.

PLATE II

FIGS. 7 and 8. Cells from different nodules, showing differential staining of minute granules. Flemming's and Altmann's fixation, respectively.

FIG. 9. Cell from nodule after 12 hours treatment with 95 per cent alcohol before fixation. Murray's fixation.

FIGS. 10 and 11. Cells from different nodules after 16 hours treatment with 95 per cent alcohol before fixation. Murray's fixation.

FIG. 12. Cell from a section of a nodule after treatment with 95 per cent alcohol before fixation. Altmann's fixation.

FIG. 13. Section from nodule, showing minute granules. Altmann's plus 5 per cent of acetic acid fixation.

FIG. 14. Section of nodule after 12 hours treatment with ether before fixation. Champy-Kull's fixation.

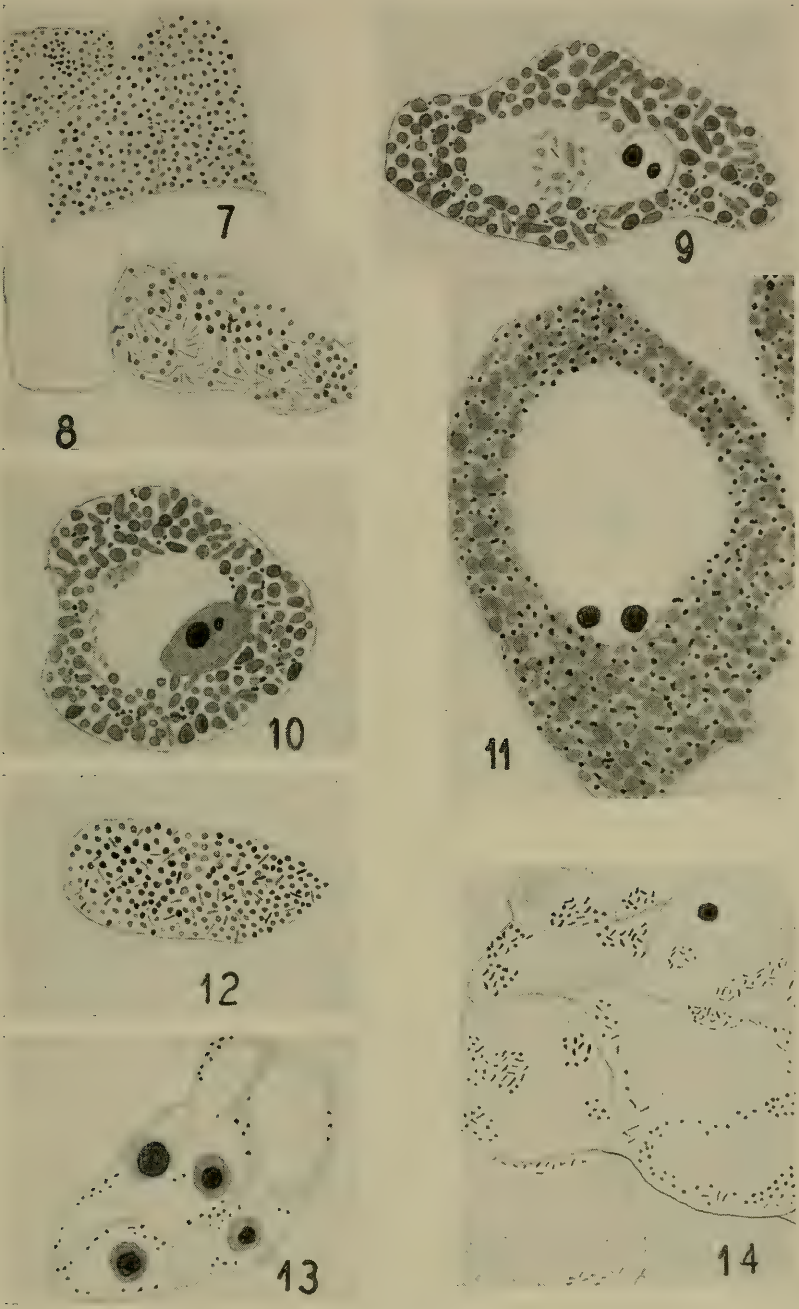
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PLATE II

Figure 13 (Plate II) represents a specimen from a nodule which was fixed in Altmann's fluid to which 5 per cent of acetic acid was added, and stained with anilin-fuchsin and methyl green.

In order that a more satisfactory comparison between alcohol and non-alcohol-treated specimens could be made, nodules were cut and the two halves subjected to different treatment. This procedure overcame, in a measure, the difficulties encountered when examining different nodules. Sections of parts of a single nodule mounted on slides in close juxtaposition facilitated a detailed comparative study. Likewise, there remained slight opportunity for variations in fixing and staining procedure. Such a comparison showed a strikingly close resemblance in the rod-like and granular cell contents. In numerous cases there were no observable differences in the size, general arrangement, nor in staining qualities of these inclusions in the separate halves (Plate III, Figs. 16 and 17).

In smears of the nodule, prepared by thorough maceration and allowing them to dry before fixation, one is frequently able to observe minute rods and granules, sometimes in abundance, stained red with anilin-fuchsin and toluidin blue, or methyl green (Plate III, Figs. 18, 19, and 20). The same is true if the smear has been treated with alcohol or ether prior to mitochondrial fixation (Plate III, Fig. 22). Similar forms of bacteria possessing identical staining qualities occur on artificial media which had been inoculated with substance from the nodule or from the soil. Frequently, these minute bacteria from artificial media stain red while larger forms occurring in the same media stain blue or green (Plate III, Figs. 21, 23, and 24).

PLATE III

Fig. 15. Section of a nodule different from the one shown in Fig. 14 after 12 hours treatment with ether before fixation. Champy-Kull's fixation.

Fig. 16 (*a*) and (*b*). Cells from approximately the same region of the same nodule: (*a*) after 12 hours treatment with 95 per cent alcohol before fixation, and (*b*) without alcohol treatment. Murray's fixation.

Fig. 17 (*a*) and (*b*). Cells from approximately the same region of the same nodule: (*a*) after 12 hours treatment with 95 per cent alcohol before fixation, and (*b*) without alcohol treatment. Champy-Kull's fixation.

Fig. 18. Bacteria from nodule smear. Schaudinn's fixation and stained in iron-hematoxylin.

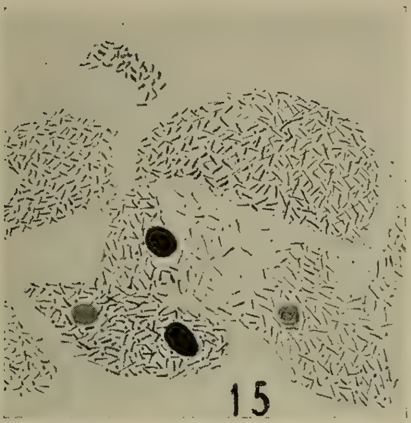
Fig. 19. Bacteria from smear made from a nodule. Altmann's fixation.

Fig. 20. Bacteria from nodule smear. Champy-Kull's fixation.

Figs. 21 and 23. Bacteria from different nodules and cultured on lithium-agar plate. Altmann's fixation.

Fig. 22. Bacteria from nodule smear, dried and treated with 95 per cent alcohol. Altmann's fixation.

Fig. 24. Bacteria from mannite medium inoculated from the soil. Champy-Kull's fixation.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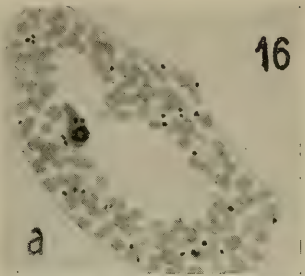
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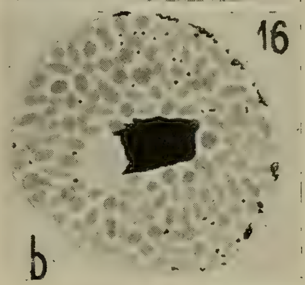
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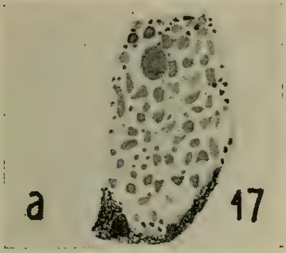
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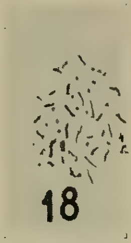
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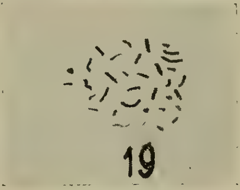
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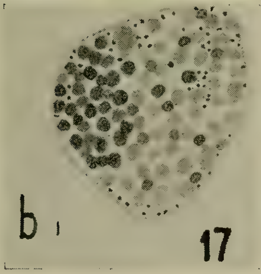
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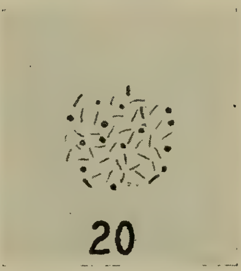
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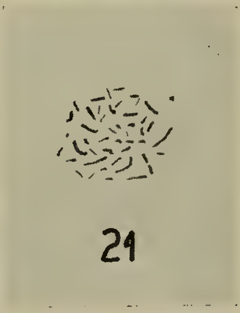
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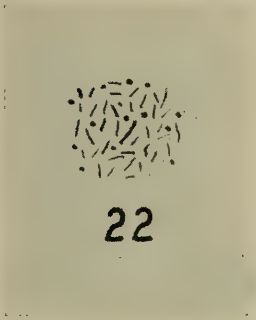
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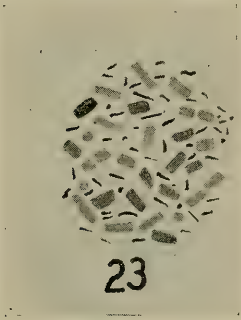
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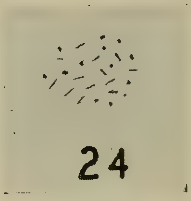
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PLATE III

Freshly macerated nodules, in addition to showing large, mature forms of bacteria, also show many minute rod-like and granular forms. Many, due to their minute size, presented a dark appearance. Supravital tests on this material with Janus green revealed nothing definite. There were no indications that the number of dark staining rods and granules had been increased.

Worthy of note at this point is the fact that among the different mitochondrial methods of technique, Regaud's method gave the least satisfactory results, following treatment of the nodule with alcohol. While granules and a few rod-like inclusions frequently appeared in cells after alcohol treatment, they did not stain well nor stand out nearly so clearly as after other methods of fixation nor in cells which had not been subjected to alcohol. There were some indications that granular material in the alcohol-treated nodules represented merely precipitate, and, for that reason have been omitted from the list of illustrations.

Discussion

Topley and Wilson (1932, p. 315) describe the nodule organism, *Rhizobium radicicola,* as one having a life-cycle with gross changes in morphology: " Shows non-motile coccoid forms; very small, highly motile, ellipsoidal form (swarmer), 0.9 × 0.2 microns; motile rods 2–3 × 0.5 microns." They state with respect to the smaller forms, p. 314: " They are so small as to be able to pass through a Chamberland filter." Löhnis (1921), (Plate 15, Fig. 195; Plate E, Fig. 16; Plate R, Fig. 83), gives an excellent idea of the minuteness and variation in size of rod-like bacteria belonging to *B. radicicola.* It has been thought by some investigators that other microörganisms, besides *B. radicicola,* may enter the nodule of the clover. To quote Wallin (1927), p. 90: " In the young root nodules no other organisms but the *Bacillus radicicola* are present in the cells. As the nodules mature, other microörganisms begin to invade the cells of the nodule. In an old nodule, very few if any *Bacillus radicicola* can be found in the nodule. They appear to have been replaced by a variety of parasitic microörganisms."

In the preceding pages I have shown that minute bacteria, both rod-like and granular, present in the nodule and in the soil are not unlike, in their general appearance and staining properties, rod-like inclusions of the clover nodule and described heretofore as mitochondria. Likewise, the illustrations show how mitochondria-like inclusions appear frequently in nodules which have been subjected to prolonged treatment with alcohol, ether, or fixatives containing acetic acid. Since, in addition, minute bacterial forms of *B. radicicola* enter the clover rootlets and subsequently bring about the formation of the nodule, together with the probability that still other bacterial microörganisms invade the nodule, it appears likely that the minute inclusions having a mitochondrial appearance represent bacteria. For the fact that among nodules, or halves of nodules, which have received different treatment prior to fixation with mitochondrial fixatives (excepting perhaps Regaud's), there have existed in many cases no observable differences in the nature of minute cell inclusions, strongly indicates that inclusions lacking essential mitochondrial characteristics are present in the nodules of the clover.

A close study of the illustrations given by Cowdry, showing the morphological forms of *B. radicicola* as they occur in the clover nodule, leads me to believe that the minutest of the bacterial forms, such as I have observed in smears of nodules, freshly macerated nodules, as well as from cultures of bacteria from the nodule and from the soil, have not been included in these illustrations as bacteria, but as mitochondria. The reason for such an omission, if true, appears to lie in the fact, largely, that Cowdry is of the opinion that the smallest of bacterial forms found in the nodule stain green with methyl green after mitochondrial fixatives and not red with anilin-fuchsin. He states, p. 340: "A little farther up the rootlet small forms of *B. radicicola* are encountered, as represented in figure 3. They are distinguishable from the mitochondria in the preparations by their green color, their larger size, and their variable distribution in clumps in different cells." As has already been shown in preceding pages and the illustrations, bacteria equaling in size apparent mitochondria as observed in nodules, occur in nodules and in the soil and stain red with anilin-fuchsin and methyl green or toluidin blue. Many of these bacteria are apparently smaller than many red colored rods and granules observed in nodules which may or may not have been treated with lipoid solvents before fixation.

For the reason that differential staining sometimes occurs among these inclusions, whether in the nodule, smears, or on slides prepared from cultures of material from the nodule and from the soil, it would appear that the slightest variation in the chemical composition of the inclusions, or in technique, is often all that is necessary to alter the staining reaction of these minute forms. For the fact that, in numerous cases, where nodules have been subjected to alcohol treatment, cells occur which are completely filled, or nearly so, with minute rods and granules, some of which stain red while others stain green, makes it quite obvious that minute bacteria within the cell of the nodule may stain like mitochondria.

From the above observations it would seem that there are three possible conclusions. Firstly, either mitochondria as such do not exist in these nodules, but have been confused with an "invading form" of minute bacteria from the soil; or secondly, mitochondria possess characteristics which have not been fully recognized, in that they may withstand alcohol, ether, dilute acetic acid, maceration of the nodule, and drying; or thirdly, they are indistinguishable in the nodule from minute forms of bacteria with our present-day methods of technique. Certainly, the above methods of technique have aided little in making a clear distinction between bacteria and mitochondria, but in the case of the clover nodule the results materially strengthen the indications that the alleged mitochondria are merely forms of minute bacteria.

Conclusions

1. The morphology of minute cytoplasmic inclusions as they occur in the nodule of the clover, their response to Janus green and lipoid solvents, staining reaction, fail to produce sufficient evidence for placing them in the category of mitochondria.

2. Bacillary and granular bacteria occur in clover nodules, in nodule smears, in cultures of material from nodules and from the soil on artificial media, which possess staining qualities and physical features characteristic of mitochondria.

3. Minute rods and granules, having the physico-chemical properties of mitochondria, can be demonstrated in some nodules which have been subjected to prolonged treatment with 95 per cent alcohol, ether and fixatives containing 5 per cent of acetic acid.

4. Differential staining cannot be relied upon in this case to distinguish bacteria from the alleged mitochondria, since certain forms of minute bacteria found in nodule-smears and cultures made from the nodule and from the soil stain red with anilin-fuchsin and methyl green after mitochondrial fixatives, just as do many rod-like and granular inclusions in sectioned material.

5. Indications are that the slightest variation in technical manipulations, or possibly chemical composition of different regions of the nodule, is often sufficient to alter the staining reaction of otherwise identical minute forms occurring in the clover nodule.

6. Janus green apparently does not stain any of the minute rods nor granules found in freshly macerated nodules.

7. The results of this investigation appear to support strongly the inference that minute bacterial forms have heretofore been misinterpreted as mitochondria in the nodule of the clover.

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THE RELATION BETWEEN LUMINOUS INTENSITY, ADAPTATION TO LIGHT, AND RATE OF LOCOMOTION IN AMOEBA PROTEUS (LEIDY)

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Introduction

Strasburger (1878), Loeb (1890), and Davenport (1897) maintain that organisms appear to move faster in strong light than in weak light because their path is straighter and orientation is more precise and they conclude that the rate of locomotion is independent of intensity. This conclusion is supported by the results obtained by Mast (1910, 1911) on *Amœba proteus* and blow-fly larvæ, Dolley (1917) on *Vanessa antiopa,* Minnich (1919) on *Apis mellifera,* Mast and Gover (1922) on *Phacus pleuronectes* and *Euglena gracilis,* Folger (1925) on *Amoeba proteus,* and Ullyott (1936) on *Dendrocœlum lacteum.*

But it is not supported by those obtained by Oltmanns (1892), Holmes (1903), and Laurens and Hooker (1920) on *Volvox,* Yerkes (1903) on *Gonionemus,* Herms (1911) on blow-fly larvæ, Patten (1917) on the whip-tail scorpion, Buddenbrock (1920) on *Helix,* Moore and Cole (1921) on the Japanese beetle and *Drosophila melanogaster,* Clark (1928) on *Dineutes assimilis,* and Welsh (1932) on *Unionicola.*

Clark made a more thorough study of the problem than any of the other investigators. He found that with increase of intensity, the rate of locomotion increases to a maximum at 0.5 m.c., then remains constant until 8,000 m.c. is reached, and then decreases. Trezise (1936) obtained similar results in observations on *Dineutes nigroir.*

Mast (1907, 1927) contends that the activity of organisms in any given luminous intensity is closely correlated with the state of adaptation of the organism to that intensity. This contention is supported by results obtained by Walter (1907) in observations on *Planaria gonocephalus.* He maintains that this organism moves faster in light than in darkness, but the results which he presented show that if the specimens are transferred from darkness to light and left there, the rate of locomotion decreases so that after 10 minutes it is much lower than it was in darkness.

It is consequently obvious that there is great diversity in the results obtained by different investigators concerning the relation between rate of locomotion and luminous intensity. This is doubtless largely due to the fact that in most of the investigations the state of adaptation was not adequately considered.

MATERIAL AND METHODS

Amoeba proteus (Leidy) was used exclusively in the following experiments. It was raised in finger-bowls containing a few grains of rice and numerous chilomonads in Hahnert solution, which consists of pure water (1 liter), $CaCl_2$ (4 mg.), $NaHCO_3$ (4 mg.), $CaH_4(PO_4)_2$ (2 mg.), KCl (4 mg.), $Mg_3(PO_4)_2$ (2 mg.). The cultures were kept at a temperature of 24°–26° and at a luminous intensity of about 20 meter-candles.

TABLE I

Relation between rate of locomotion in *Amoeba proteus* and intensity of light. The "total average rate" is the mean for all measurements at the indicated intensities of light. The "mean maximum rate" is the mean for all measurements made after the maximum rate was reached. The time required to reach the maximum rate was obtained by inspection of the graphs for each intensity of light. The standard deviation was calculated for the rate after the maximum was reached.

Intensity	Number of specimens used	Total average rate	Mean maximum rate	Time required to reach maximum	Percentage of measurements on lobose forms	Standard deviation
meter candles		*micra/min.*	*micra/min.*	*minutes*		*micra/min.*
50	21	128.8	128.8	1	7.4	10.8
225	18	124.8	131.0	11	8.7	9.0
4,170	14	145.8	147.4	16	16.5	8.6
7,530	16	160.0	186.6	14	9.0	11.3
11,120	18	176.2	198.4	11	12.0	9.3
13,140	18	191.3	215.7	15	10.8	6.4
15,000	19	198.1	219.3	8	16.8	11.4
17,000	20	176.0	211.1	17	10.8	11.4
20,000	23	170.0	200.2	22	14.7	4.3
24,000	22	160.9	205.7	23	12.4	14.2
26,600	21	151.8	195.6	22	17.2	9.8
40,000	23	120.7	150.2	30	20.4	8.5

All the experiments were performed in a darkroom. The light used was produced by a monoplane filament stereopticon bulb mounted in a projection lantern which was in turn enclosed in a box, light-tight, except for an opening 25 cm. square. A parallel-sided glass vessel 8 cm.

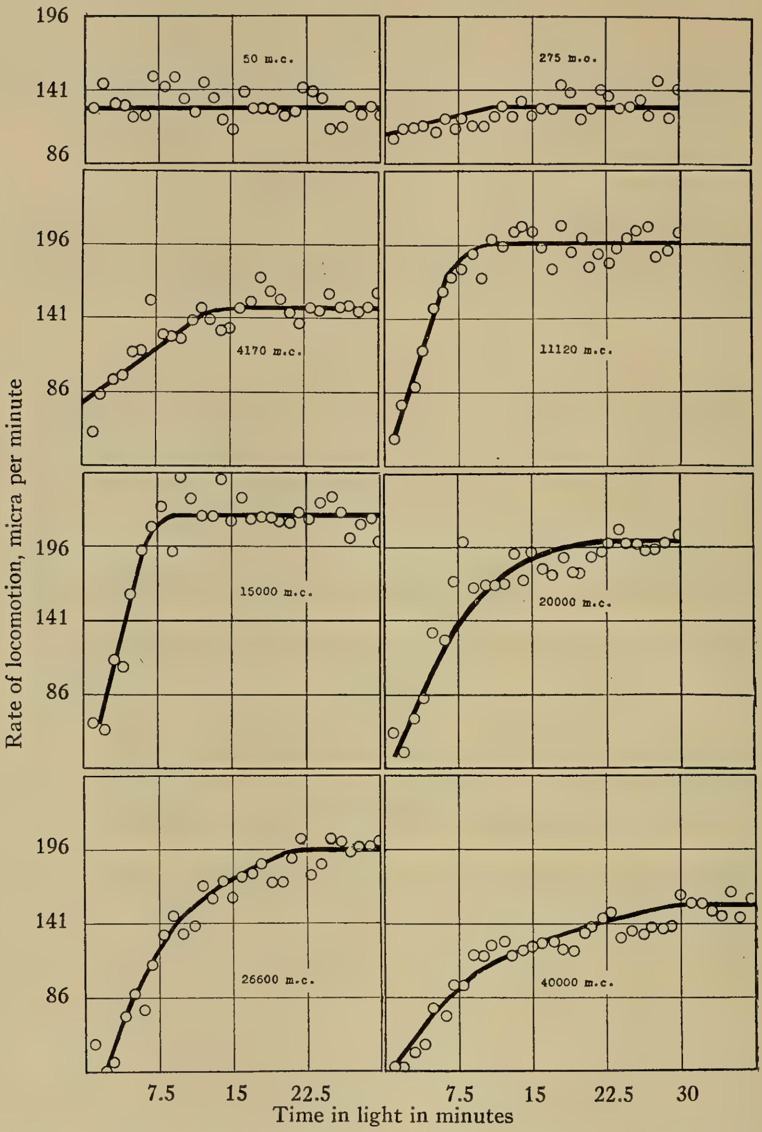

FIG. 1. Relation between adaptation to light of different intensities and rate of locomotion in *Amoeba proteus*.

Each point in the figures represents the average for one measurement on each of 14 to 23 specimens (see Table I). The time in light is the time from the beginning of movement after exposure until the measurements were made.

thick, filled with distilled water, was placed in the box in front of the lamp, to absorb the heat waves.

Three stereopticon bulbs were used (300, 500, and 1,000 W., 120 V.). The voltage on the line was maintained at 110 ± 2 volts by means of a rheostat. The desired intensities of light were obtained by means of these bulbs and Eastman Kodak neutral-tint filters.

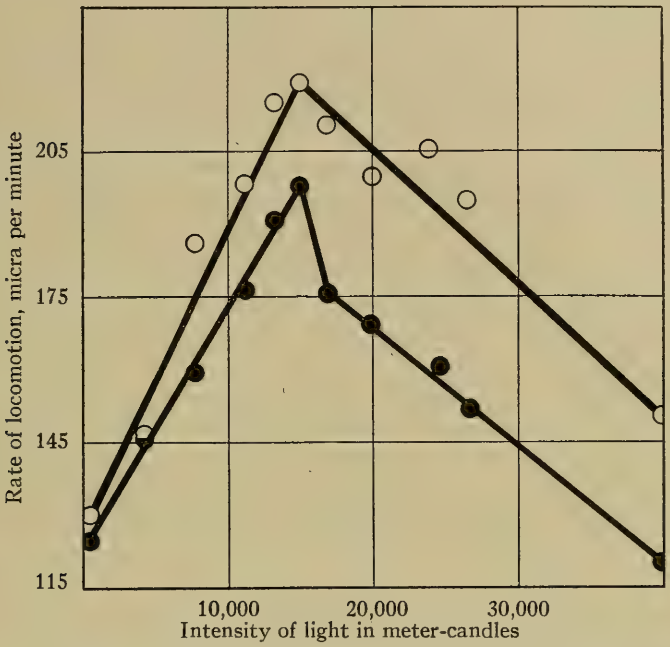

FIG. 2. Relation between intensity of light and rate of locomotion in *Amoeba proteus.*

Upper curve, mean maximum rate; lower curve, total average rate.

Each point on the lower curve is the average for 420 to 920 measurements on 14 to 23 specimens. Each point on the upper curve represents the average for the same number of measurements in the lowest intensity but progressively fewer as the intensity increases (see Table I and Fig. 1).

The beam of light was reflected up through the stage of a compound microscope by means of the substage mirror. The microscope had no substage condenser. The intensity on the stage was measured directly by means of a Weston photometer.

A Pitts warming stage (Pitts, 1932) was fastened to the stage of the microscope. This consists essentially of a beaker in a metal box so arranged that water of any desired temperature can be passed through the box and around and under the beaker.[1] The bottom of the beaker consisted of smooth Pyrex glass.

The observations were made as follows: A large number of amœbæ were taken from a vigorous culture and put into a small glass dish containing 10 cc. redistilled water and left ten minutes. The water was then changed four times. Then about 60 large specimens with few food vacuoles were selected and put into a dish containing redistilled water and left without food 15 hours or longer in darkness at a temperature of 24° to 26°. Then 5 to 10 of the specimens were transferred to a dish containing .001 N $CaCl_2$ and immediately transferred again, with $CaCl_2$ solution, to a vaseline enclosure on the bottom of the beaker in the Pitts warming stage and covered with a cover-glass. The solution in the enclosure was so deep that the amœbæ did not touch the cover-glass. The temperature in the beaker was maintained at $24 \pm 0.5°$.

They were then subjected to light of 50 m.c. for five minutes, then to darkness for 30 minutes. Then a weak red light was turned on and a specimen which was monopodal or nearly so was selected, brought to focus, and exposed to white light of the desired intensity, from the projection lantern.

The posterior end of the image of the amœba, projected on black paper by means of a camera lucida, was then outlined with a yellow pencil, once a minute for 30 or 40 minutes, and the rate of locomotion calculated. If the specimen under observation came in contact with other specimens or with the vaseline ring during this time the record was discarded. The whole process was then repeated with other specimens in light ranging from 50 to 40,000 meter candles. The results obtained are presented in the following paragraphs, Table I, and Figs. 1 and 2.

Results

In all but the lowest intensities movement ceased shortly after the specimens were exposed, and in the highest intensities it did not begin again until 2–7 minutes later. In these intensities movement usually began in a pseudopod on the upper surface of the amœba. This pseudopod often extended until it contained nearly all the substance in the amœba, then a pseudopod formed at the base and extended in contact with the slide. This frequently occurred several times during the process of adaptation to light.

In the lower intensities the specimens were fairly consistently and firmly attached to the substratum and monopodal in form. In the

[1] Small pieces of glass 5 mm. thick were cemented to the inner base of the warming stage so that a constant depth of water under the beaker obtained.

higher intensities they were much less consistently monopodal and attachment to the substratum was more sporadic.

Figure 1 shows that, in all except the lowest intensity, as the time of exposure to light increased, the rate of locomotion rapidly increased to a maximum and then remained constant; and that, as the intensity increased, the time required for the rate to reach maximum decreased from about 11 minutes at 225 m.c. to a minimum of about 8 minutes at 15,000 m.c., and then increased to about 30 minutes at 4,000 m.c. This proves that the rate of locomotion in *Amoeba proteus* is closely correlated with the state of adaptation to light and it indicates that, except in the very low intensities, the time required for light-adaptation is least at 15,000 m.c.

Table I and Fig. 2 show that as the intensity to which the amœbæ were exposed increased, the rate of locomotion after the amœbæ were fully light-adapted, increased from 128.8 ± 10.8 micra per minute at 50 m.c. to a maximum of 219.3 ± 11.4 micra per minute at 15,000 m.c., and then decreased to 150.2 ± 8.5 micra per minute at 40,000 m.c. This proves that the rate of locomotion in *Amoeba proteus* is very definitely correlated with the intensity of the light, and that the optimum intensity of light is 15,000 m.c. Figure 2 shows also that the total average rate is correlated with the intensity of the light in essentially the same way as the mean maximum rate.

Table I shows that the percentage of lobose forms increased with increase in luminous intensity, and that the standard deviation for the mean maximum rate is relatively low.

The average rate of locomotion of 20 amœbæ for 30 minutes each in the red light used in the experiments described above, was 134.7 ± 8.9 micra per minute. There was no indication of decrease in rate in any of the specimens after exposure to the red light. The intensity of the red light was not measured but the rate of locomotion in it was somewhat higher than the rate of locomotion in white light of 225 m.c. in which there was marked retardation (Fig. 1). The facts that the rate in red light (in which there was no retardation) was higher than the rate in white light (in which there was marked retardation) and that blue is very much more efficient than red in inducing retardation (Harrington and Leaming, 1900 and Mast, 1910) strongly indicate that retardation and decrease in rate of locomotion with increase in intensity (in the higher intensities) observed in white light, is due largely to the action of blue and other short waves, and that increase in rate of locomotion with increase in intensity (in the lower intensities) is due largely to the action of red and other long waves.

There is no evidence which indicates the nature of the action of the longer waves of light, but it is well known that the shorter waves tend to induce gelation. It is therefore highly probable that retardation in rate of movement is due to the gelating effect of these waves.

SUMMARY

1. The rate of locomotion in *Amoeba proteus* is definitely correlated with the intensity of the light to which it is exposed and the state of adaptation.

2. In light of any given constant intensity, as adaptation to light increases, the rate of locomotion increases to a maximum and then remains constant, but in constant light of different intensities, the time required for adaptation decreases from about 15 minutes at 225 m.c. to a minimum of about 7 minutes at 15,000 m.c. and then increases to about 30 minutes at 40,000 m.c. and the rate of locomotion increases from 128.8 ± 10.8 micra per minute at 50 m.c., to a maximum of 219.3 ± 11.4 micra per minute at 15,000 m.c., and then decreases to 150.2 ± 8.5 micra per minute at 40,000 m.c.

3. Increase in rate of locomotion with increase in intensity to optimum at 15,000 m.c. is largely due to some unknown action of the longer waves of light. Decrease in rate with increase in intensity beyond the optimum is probably due to the gelating action of the shorter waves of light.

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THE RÔLE OF THE HYPOPHYSEAL MELANOPHORE HORMONE IN THE CHROMATIC PHYSIOLOGY OF FUNDULUS[1]

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The endocrine control of melanophore reactions of vertebrates has been extended within the past twenty years to the majority of animals which exhibit color changes. It has long been generally conceded that the pituitary gland is the sole regulator of chromatic activity in the Amphibia (Smith, 1916; Allen, 1916; Atwell, 1919; Swingle, 1921; Hogben, 1924). Within recent years, the cyclostomes (Young, 1935), the elasmobranchs (Lundstrom and Bard, 1932), and certain reptiles (Noble and Bradley, 1933; Kleinholz, 1936) have been found to react to hypophysectomy by complete pallor due to the concentration of the pigment in the cutaneous melanophores. It is also generally acknowledged that such hypophysectomized specimens remain indefinitely pale regardless of certain environmental conditions which in normal specimens would induce melanophore expansion. Furthermore, these hypophysectomized specimens cannot normally exhibit the dark phase of their coloration unless the melanophore hormone of the pituitary is administered in one form or another to them.

The teleosts are, as far as I am aware, the only color-changing vertebrates thus far investigated which do not behave to hypophysectomy like those already mentioned. The melanophores of the teleosts have long been known to be controlled by the sympathetic nervous system (von Frisch, 1911), but the importance of the pituitary gland has not been satisfactorily evaluated except perhaps in one case, *Ameiurus* (Parker, 1934*a*; Abramowitz, 1936).[2] In *Fundulus*, the relation between the pituitary and the chromatic function is uncertain. Parker (1935) is of the opinion that the hypophysis is without functional significance as a normal means of controlling color change in *Fundulus*, although later (1936) he writes that certain responses call for further

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[1] Aided in part by a grant from the Rockefeller Foundation, administered by F. L. Hisaw.

[2] Recently Veil (1937) has reported that *Ameiurus* reacts to hypophysectomy as the selachians do. Her paper is probably a preliminary report since very little or no data are given in support of this conclusion.

elucidation. This paper is intended to define the rôle of the hypophyseal melanophore-expanding hormone in the chromatic physiology of *Fundulus.*

Desmond (1924) was apparently the first to investigate the effect of the pituitary on the melanophore responses of *Fundulus,* but his work has been overlooked by the subsequent investigators of this problem. He found that hypophysectomy was without effect on the melanophore reactions of young *Fundulus.* This negative effect following hypophysectomy could not be attributed to the absence of the melanophore hormone in the pituitary of *Fundulus,* for he found that while pituitary transplants from adult fish or amphibians into *Fundulus* were without effect on the melanophores of the host, similar transplants made into hypophysectomized axolotls or tadpoles produced the typical darkening of these hosts. It thus appeared that the pituitary of *Fundulus* contained the melanophore-expanding hormone which was effective on amphibian melanophores but ineffective on its own black pigment cells. Matthews (1933) also observed that hypophysectomy did not affect the melanophores of adult *Fundulus.* The melanophores expanded normally when pituitaryless specimens were adapted to a black background and contracted promptly when adapted to a white background. However, when Matthews immersed isolated scales in a water extract of *Fundulus* pituitaries, the scale melanophores always contracted. On the belief that the pituitary contained a melanophore-contracting hormone, Matthews reasoned that it might conceivably affect the contraction of denervated melanophores in a caudal band (Mills, 1932). However, denervated trunk or tail areas faded in the same time in hypophysectomized as in intact animals during prolonged white-background adaptation.

Thus the situation became complicated, for Matthews' work indicated that a contracting hormone was present in the pituitary of *Fundulus,* and yet the melanophores, innervated or denervated, contracted normally in the absence of the pituitary. The presence of a contracting principle was disputed by Kleinholz (1935), who showed that *Fundulus* pituitaries could darken normal white-adapted frogs and catfishes, and hypophysectomized lizards, and furthermore that injections of pituitrin or extracts of crushed *Fundulus* pituitaries were followed by a darkening of a pale denervated caudal band in normal white-adapted *Fundulus.* The latter experiment is significant in relation to the neurohumoral theory (Parker, 1932) for it raises the possibility that the black background response (the expansion of contracted denervated melanophores in a normal *Fundulus* when adapted to a black background) might be due, not to a dispersing neurohumor as Parker

(1934*b*) has maintained, but to the melanophore-expanding hormone of the pituitary (Kleinholz, 1935).

The responses of teleost melanophores to various hypophyseal extracts are by no means clarified. Spaeth (1917), Hewer (1926), Odiorne (1933) and Matthews (1933) have reported melanophore contraction following treatment with certain hypophyseal extracts. The significance of these reports is enhanced by Hogben and Slome's (1936) recent work with amphibians where, in *Xenopus,* evidence for a dual pituitary control by melanophore-expanding (" black ") and contracting (" white ") hormones has been accumulated. Thus it may be possible that the pituitary gland of *Fundulus* contains a contracting melanophore principle. The existence of a melanophore-expanding hormone in the teleost pituitary rests on a more substantial basis, for this principle has been shown to be present in the pituitary glands of all chordates from cyclostomes through the primates, including man. The complexity of melanophore responses to pituitary extracts was increased by the work of Przibram (1932), who showed that the injected dosage was a factor determining the direction of melanophore response. He found that low dosages of a commercial pituitary extract resulted in an expansion of amphibian melanophores, while when higher dosages were employed the melanophores contracted.[3] This result he offered as an explanation of the conflicting reports of the effect of pituitary extracts upon the melanophores of the same fish (*Phoxinus*) by Abolin (1925) and by Hewer (1926). There is thus a great difference of opinion regarding the responses of teleost melanophores (intact, isolated from the body, or isolated experimentally—denervated—within the body) to various pituitary extracts.

METHODS

Large healthy specimens of *Fundulus heteroclitus* were used throughout this work. A stock supply of fish was maintained in a large aquarium of running sea water and fed daily with generous portions of macerated clams and live shrimp. Background responses were elicited by the customary white or black vessels, three of each kind being placed on a lead table and arranged so that a constant current of sea water flowed through all the dishes. All six vessels were illuminated by two 60-watt lamps. Hypophysectomies were performed by the opercular approach (Abramowitz, 1937). Slightly over 200 hypophysectomies were performed and an equal number of normal specimens kept as controls. Since the operator can determine whether the entire gland is being removed, histological examination of the hypothalamus was deemed unnecessary. When the gland was seen to fragment during an operation, the animal was immediately discarded. Twelve hypophysectomized *Fundulus* were selected at random and the region of the hypothalamus dissected under an operating binocular but no trace of pituitary tissue could be found. Denervating operations in the base of the tail were carried out by Wyman's technique (1924). The width of these bands was in every case 2 mm., since all cuts were made with the same instrument. The technique of extracting the melanophore-expanding hormone from the blood was somewhat similar to that described by Jores (1933) for man.

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[3] Przibram's experiments were carried out with a commercial pituitary extract (Infundin), which is not primarily prepared for the melanophore hormone and which is probably contaminated with preservatives, and other posterior pituitary hormones. When high concentrations of Infundin were employed, the presence of the extraneous substances might have conceivably complicated the response. My results with hypophysectomized frogs do not substantiate Przibram's explanation for many such specimens injected with high dosages of a relatively pure melanophore hormone (1 gram equivalent of whole sheep pituitary powder potentiated 25 times by alkalinization) always became black and remained so for two weeks.

RESULTS

I. Effect of Hypophysectomy on the Responses of Denervated Melanophores to Backgrounds

A denervated band of melanophores was established in the base of the tail of each of 26 *Fundulus*. Twelve of these specimens were hypophysectomized, and these and the remaining 14 unoperated animals placed in illuminated white vessels. Three days later the denervated bands which during this time appear black due to melanophore expansion become completely pale (melanophore contraction) in both unoperated and hypophysectomized groups. The pituitary then does not affect the contraction of denervated melanophores during prolonged white background adaptation immediately following denervation, as first demonstrated by Matthews (1933). Then, both sets were transferred to an illuminated black background. Twelve hours later, 11 of the 14 normal animals exhibited fully dark bands; the remaining 3 showed partly dark bands. In the hypophysectomized series, 6 showed faintly dark bands and 5 exhibited completely pale bands.[4] After 36 hours of black-background adaptation, there was no change in either of the two series of animals.

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[4] In all of the experiments cited, microscopical examination of the melanophores was made. In the absence of a quantitative treatment of melanophore responses, the following descriptive terms will be employed: fully dark—complete melanophore expansion; partly dark—slightly more expanded than intermediate; faintly dark—melanophores more contracted than intermediate; pale—complete melanophore contraction.

Thus the pituitary seems to be indispensable for the complete expansion of denervated melanophores following black-background adaptation. Since this experiment is important from the standpoint of the neurohumoral theory (Parker, 1932) it was repeated in different ways. Nineteen *Fundulus* were hypophysectomized, and denervating operations induced in their caudal fins. These were placed on a white background for 13 days. By this time, the cut nerves in the tail fin have completely degenerated (Parker and Porter, 1933; Abramowitz, 1935). The bands are also completely pale due to extreme melanophore contraction. Thirteen additional animals to serve as control were treated in the same way except that they were not hypophysectomized. At the end of 13 days, both sets were transferred to a black background for 24 hours. The control animals showed fully black bands but in the hypophysectomized group, only 1 band was fully black, 12 were faintly dark, and 6 completely pale.

This black-background experiment was again repeated in a third manner. In 30 *Fundulus,* caudal bands were established and the animals white-adapted for 5 days. Ten animals were then changed to a black background for 24 hours. The remaining twenty were hypophysectomized and also transferred to a black background for 24 hours. All 10 normal animals exhibited fully dark bands. In the hypophysectomized group, 11 showed faintly dark bands, 1 fully dark, and 8 completely pale. The experiment was continued, and the specimens examined after 40 hours of black-background adaptation. The normal group still showed fully dark bands. Half of the hypophysectomized evidenced faintly dark bands, and half showed completely pale bands.

Thus, it appears that the melanophore (expanding) hormone of the pituitary is necessary for complete expansion of denervated contracted melanophores in the change from white to black background. In this respect, it is in agreement with *Ameiurus* (Abramowitz, 1936). So far nothing has been said of the reactions of the innervated melanophores following hypophysectomy. In all of the experiments, the innervated melanophores contracted promptly when the fish was exposed to a white background in both normal and hypophysectomized. Both sets of animals also showed expansion of the innervated melanophores when exposed to a black background. These statements are confirmatory of Matthews' observations. However, my hypophysectomized animals never became quite as dark as normal animals when exposed for equal periods of time to a black environment.

These experiments, as well as those of Matthews, indicate that the hypophysis has no effect on the contraction of either innervated or denervated melanophores. The latter must be due to some factor (neurohumoral) other than nervous action or a possible hypophyseal melanophore-contracting hormone. The full expansion of contracted denervated cells, however, does not occur in the absence of the melanophore-expanding hormone, and the expansion of the innervated melanophores is only slightly retarded in hypophysectomized animals. It would seem, therefore, that Parker's conception of the neurohumoral theory as applied to *Fundulus* may be modified to include the aid of the melanophore-expanding hormone of the pituitary.

II. Presence of the Melanophore (Expanding) Hormone in the Blood of Fundulus

If these results are correct it should be possible to obtain the melanophore hormone from the blood of *Fundulus*, especially the black-adapted specimens. Previous attempts to detect the presence of hormones in the blood of *Fundulus* have been unsuccessful. Mills (1932) injected blood from a dark *Fundulus* into a pale one, but without effect on the melanophores of the latter. The reverse experiment, blood from a pale fish injected into a dark one, was equally negative. Parker (1934*b*) also reported similar experiments and similar negative results. Obviously, these experiments indicate that the blood of a dark *Fundulus* is ineffective in eliciting expansion of the innervated melanophores of a pale animal, but they do not prove that the blood of *Fundulus* does not contain the melanophore-expanding hormone. The fact that blood of a dark fish does not cause a pale fish to darken is due, as shown in the next section, to the insensitivity of the innervated melanophores of *Fundulus* to the melanophore hormone. Consequently, the presence of the latter could not be demonstrated by the experiments already cited.

The test was therefore made on the hypophysectomized frog, which in my experience is the most reliably sensitive object for the biological detection of the pituitary melanophore hormone. Specimens of *Rana pipiens* were totally hypophysectomized by a direct oral route, and within the following three hours these specimens became totally pale. Hypophysectomized males (30–40 grams in weight) were used throughout. Blood was drawn from 10 black-adapted specimens and extracted with 10 cc. of 50 per cent ethanol by boiling for 2–3 minutes. The soluble portion was separated by centrifugation, dried, extracted with 70 per cent ethanol. The soluble portion was separated, taken up in 0.5 cc. distilled water, and injected into the dorsal lymph sac of hypophysectomized frogs. These became dark within an hour and remained dark for 5 hours, after which they became again pale. A similar test with the blood of 10 white-adapted fish also gave a positive response but less intense, inasmuch as the frogs remained dark for 3 hours. Repetition of the experiment with blood from white-adapted hypophysectomized or black-adapted hypophysectomized specimens (hypophysectomized 7 days previously) did not produce any response in the pituitaryless frogs.

III. Quantitative Studies on the Melanophore Hormone of the Pituitary of Fundulus

Having demonstrated the presence of the melanophore-expanding autocoid in the blood, and the inability of the denervated melanophores to expand fully in the absence of this hormone from the circulation, it was necessary to determine quantitatively whether the pituitary could elaborate sufficient hormone to affect the denervated cells. Thirty white-adapted hypophysectomized specimens containing pale denervated bands were injected intraperitoneally in groups of 6 animals, each with various known amounts of melanophore hormone prepared from commercial sheep pituitary powder. All experiments in this section with sheep melanophore hormone were performed with dilutions of the same stock solution. Quantitative treatment is obtained by expressing the injected dosage in terms of gram equivalents of commercial pituitary powder. Fish of equal weight (approximately 10 grams) were chosen and the specimens after injection were, of course, returned to the white background for the duration of the experiment. The minimal dosage necessary to darken a pale band was found to be 1 mg. equivalent. In no case did the innervated cells expand although as much as 1 gram equivalent was injected. Doses higher than this amount were sometimes followed by death, and the usual darkening of the entire animal. Thus the melanophores of a white-adapted fish, maintained in a continually contracted state by efferent motor impulses from the C.N.S., seem to be absolutely insensitive to the melanophore-expanding hormone. Intraperitonal injections of various known dilutions of the same stock solution into hypophysectomized frogs disclosed that the minimal dose necessary to cause the beginning of melanophore expansion in the web of the legs was 0.01 mg. equivalents. The minimal effective dose for the denervated tail melanophores of *Fundulus* is thus 100 times greater than that for the melanophores of the frog. This difference in sensitivity is especially marked when the weights of the two animals are compared (frog 35–40 grams, *Fundulus* 10 grams) and when the numbers of reacting cells are compared. The two-millimeter denervated caudal band contains roughly 7,000 melanophores, while the entire skin of the frog, although no computations were made, contains easily many times this figure. These experiments seem to explain the negative results of Mills and Parker.

The pituitary of an ordinary sized *Fundulus* (10 cm.) when extracted with distilled water contains approximately four frog units. (We define the frog unit as the amount of hormone contained in 0.2 cc., which when injected into the dorsal lymph sac of hypophysectomized frogs produces a reaction (melanophore expansion) during 3 hours. The period of 3 hours is measured as the time intervening between the time of injection and the time at which the animals regain their condition of pallor.) However, if a pituitary is extracted with a small amount of N/10 NaOH, boiled, and neutralized to pH 7 with N/10 HCl, the gland assays at 100 frog units. The potentiation of the melanophore hormone by alkali is thus about twenty-five fold, in agreement with the results of Stehle (1936). The equivalent of 1 *Fundulus* hypophysis, extracted with distilled water, is ineffective in darkening a pale denervated band of a normal white-adapted animal. However, the band may be made fully dark with the equivalent of ⅕–⅒ of a *Fundulus* pituitary when extracted with alkali. Thus there appears to be sufficient hormone in the pituitary of this fish to aid in the normal process of expansion of denervated melanophores. Similarly, blood of one dark *Fundulus,* when extracted with 50 per cent and 70 per cent ethanol, and then boiled with 1.0 cc. of N/10 NaOH, and neutralized to pH 7 is also effective in evoking a darkening of a pale band in a normal white-adapted fish. Thus, there appears to be sufficient melanophore hormone circulating in the blood of *Fundulus* to establish it as a normal agency in the chromatic physiology of this fish.

SUMMARY

1. After hypophysectomy in *Fundulus,* denervated melanophores cannot exhibit the normal black-background response. Normally innervated melanophores are only slightly affected.

2. The inability of the denervated melanophores in hypophysectomized animals to expand completely is due to the absence of the hypophyseal melanophore hormone from the blood.

3. There is sufficient melanophore hormone in the pituitary and in the circulating blood of *Fundulus* to establish it as a significant agency in the melanophore responses of this teleost.

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THE EFFECT OF ITS NITROGEN CONTENT ON THE DECOMPOSITION OF THE POLYSACCHARIDE EXTRACT OF CHONDRUS CRISPUS

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It has been frequently pointed out (Keys, Christensen and Krogh; Johnson; Waksman and Carey, et al.), that the bacterial decomposition of organic matter in stored sea water is limited by the supply of available nitrogen. A rather striking illustration of this arose from an experiment in which the organic material used was the purified polysaccharide extract of the marine alga, *Chondrus crispus,* which provides as well a natural source of nitrogen.

METHOD

Oxygen consumption was used as the index of decomposition. The customary procedure of storing glass-stoppered bottles (of approximately 200 ml. capacity) under water, at room temperature (20–25° C.), in the dark, was followed. The oxygen remaining was determined by the Winkler titration method, at various intervals. The results given are averages of at least two bottles, more often of three.

EXPERIMENTAL

In a preliminary experiment, the rate of decomposition of the extract was compared with that of the plant itself. The results, given in Table I, show the whole plant to be more readily decomposed than the extract. This fact was attributed to the lower quantity of nitrogen in the latter. For instance, plants containing 1.20 per cent nitrogen, on a dry weight basis, might yield an extract having only 0.30 per cent of nitrogen (Butler, 1936). The method of preparing the extract was described in a previous paper (Butler, 1934).

Table II shows the effect of various amounts of nitrogen, added as nitrate, on the decomposition of the extract.

It is evident from this table that the decomposition was increased in proportion to the amount of nitrogen added, up to a certain point only; other factors then apparently become limiting. In seven days the decomposition occasioned with the addition of 0.042 mgm. of nitrate nitrogen was as great as with 0.140 mgm., at least as measured by oxygen consumption. The fact that, with the lower quantity of nitrate, seven days were required to bring about the same amount of decomposition that was accomplished in three days with the larger quantity, suggests a possibility of the nitrogen being utilized more than once. This, however, is not likely, for von Brand, Rakestraw and Renn (1937) have shown that decomposition stops only after an interval varying from 8–20 days. A more probable explanation is that the bacterial population supported by the smaller quantity of nitrogen has a much lower total metabolism than that of the abundant population when nitrogen is more plentiful.

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[1] Contribution No. 144.

TABLE I

Decomposition of Chondrus crispus and its Polysaccharide Extract

Material	Mg. added per bottle	Oxygen consumed (ml. per liter)				
		1 day	2 days	3 days	5 days	6 days
Sea water only.......	—	0.09	0.24	0.36	—	0.57
Chondrus...........	3	1.11	1.71	1.89	—	4.32
"	5	—	1.26	—	3.48	—
"	10	1.38	3.60	4.32	—	5.46*
"	20	—	3.18	—	5.88*	—
Extract.............	25	0.18	0.54	0.81	—	1.47
"	5	—	0.81	—	1.92	—
"	10	0.66	1.17	1.68	—	3.36
"	20	—	1.68	—	5.31	—

* All oxygen consumed.

The bacterial numbers given in Table II were furnished by Dr. Margaret Hotchkiss at the Woods Hole Oceanographic Institution, and are simply included here as a matter of record, but will not be discussed.

Having seen the effect on the breakdown of the *Chondrus* extract of adding various quantities of inorganic nitrogen, the effect of different quantities of organic nitrogen became of interest. Since extracts from different collections of *Chondrus* contain varying amounts of nitrogen (Butler, 1936), it was a relatively simple matter to study the effect of this naturally occurring organic nitrogen on the decomposition of the polysaccharide complex. For the purpose, a series of bottles was prepared in which each group contained the extract from a different monthly collection of *Chondrus* plants. As each extract from the plants collected at monthly intervals contained a different quantity of nitrogen, a natural series was provided, in which each member had a different nitrogen content. Furthermore, this nitrogen is in that form in which it most probably occurs under natural conditions, in the plant itself.

Great care was taken to make up each extract in exactly the same concentration, 25 mgm. of extract per liter of sea water. This insured that any individual sample was directly comparable with all the others in the series. Oxygen determinations were made after one day of storage. The results are given in Table III along with the percentage of nitrogen in each sample. It is readily seen that a correlation exists between the two and is most direct where nitrogen is low. This seems, therefore, to furnish definite evidence of the limiting effect of nitrogen on the decomposition of, at least one type of, organic material such as occurs in the sea. It suggests also that the low level of nitrogen during the summer may be partially responsible for the slow rate of decomposition.

TABLE II

Effect of Added Nitrate on Decomposition of Chondrus Extract

Extract added (mg. per bottle)	Nitrate nitrogen (mg. per bottle)	Oxygen consumed (ml. per liter)		No. bacteria per ml.
		3 days	7 days	3 days
0	0	0.53	0.74	—
0	0.014	0.55	0.80	90,000
0	0.042	0.55	1.24	—
5	0	1.32	4.07	560,000
5	0.014	3.27	5.02	980,000
5	0.042	4.28	5.28*	—
5	0.140	5.28*	5.28*	3,200,000

* All oxygen consumed.

In this experiment the sea water was enriched by the addition of 1 mg. K_2HPO_4 per liter.

While in the present discussion it is assumed that the decomposition is brought about by bacteria, the possibility that the oxygen is consumed by other organisms is not excluded. The water used may, undoubtedly, have contained larger organisms. It was surface water collected in Vineyard Sound and filtered through a No. 25 net of bolting silk. The bacterial considerations cannot be discussed here although evidence was obtained for the bacterial nature of the decomposition. However, the important fact remains that the consumption of oxygen on storing the polysaccharide extract of *Chondrus* in sea water is correlated with the amount of nitrogen which it contains naturally.

TABLE III

Oxygen Consumption of Various Extracts of Chondrus crispus *

Extract	Nitrogen (per cent dry weight)	Oxygen consumption occasioned in one day by addition of extract (ml. per liter)
January	1.92	0.92
February	2.30	1.05
March	2.40	1.21
April	2.18	1.12
May	1.40	1.09
June	0.82	—
July	0.34	0.23
August	0.37	0.27
September	0.32	0.20
October	0.39	0.24
November	1.28	0.65
November	0.80	0.51
January	2.34	0.80

* The sea water used in this experiment showed an oxygen consumption of 0.55 ml. per liter in one day.

SUMMARY

The decomposition of *Chondrus crispus,* as measured by oxygen consumption, has been shown to be more readily accomplished on storing in sea water than that of its polysaccharide extract. This has been attributed to the higher nitrogen content of the former.

Inorganic nitrogen added to the polysaccharide extract of *Chondrus* increased its decomposition.

Samples of the extract containing different quantities of nitrogen have been found to decompose in direct proportion to the amount of nitrogen which they contain.

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THE DIFFERENTIAL EFFECT OF ENVIRONMENTAL FACTORS UPON MICROBRACON HEBETOR SAY (HYMENOPTERA: BRACONIDAE) AND ITS HOST, EPHESTIA KÜHNIELLA ZELLER (LEPIDOPTERA: PYRALIDAE)

III. Effect of the Sting of the Parasite and of Two Chemical Agents on the Respiratory Rate and Quotient of the Host Larvæ (E. kühniella Zeller)

NELLIE M. PAYNE

Many species of parasitic Hymenoptera paralyze their hosts by stinging them, after which they lay eggs in or near the host. Although the gross effect of the sting on the host has long been known, yet the precise effect has been little studied. In this paper the change in oxygen consumption of the host larva after being stung by the parasite is measured and these results compared with those produced by ether and by orthodichlorbenzene.

Methods and Materials

The respiratory exchange of full-grown larvæ of *Ephestia kühniella* Zeller was measured with the manometer described by Krogh (1914) and modified by Bodine and Orr (1925). Baskets of copper gauze lined with cotton were hung on the glass hook which extended from the open end of the manometer. Larvæ placed in them were unable to move about but may have developed some muscular tension by struggling against the cages. Since the larvæ tended to be sluggish when undisturbed, they probably developed but little tension. Manometers were calibrated by weighing the mercury required to fill them. They were run for periods of two and one-half hours.

Larvæ to be paralyzed were exposed to adult females of *Microbracon hebetor* Say which pierce the host larvæ with their ovipositors, then lay eggs on or near them. Since the parasite does not begin to feed on the host until these eggs hatch, the host larvæ suffer no loss of material from the parasitic attack. Occasionally a host larva will writhe and struggle when the wasp is stinging it and for some time afterwards. Stung larvæ were placed in the manometer only when they were quiet.

Larvæ were also paralyzed by ether and by orthodichlorbenzene. A dosage of the reagent that would paralyze one-half the larvæ used and from which recovery took place in at least twelve hours was chosen. Since there were wide differences between the susceptibility to chemical agents even among larvæ hatched from the eggs of the same female, considerable experimentation was required to find a suitable dose. Larvæ were given sufficient anesthetic to paralyze but not to kill them. Such an amount may be designated as the median-paralytic dose. Larvæ to be paralyzed by a chemical agent were placed in lots of twenty in a cylindrical wire cage made of screening. These cages were fastened to a hook blown onto a glass tube extending through the ground-glass stopper of a fumigation flask. The reagent was introduced into the flask through a glass tube which extended but a short distance below the stopper. Both this tube and the longer one on which the insects were hung were furnished with ground-glass stopcocks. The flasks were shaken from time to time.

After the median-paralytic dose had been established for both ether and orthodichlorbenzene, individual insects were transferred directly from the fumigating flasks into the manometers, where oxygen consumption was determined. The results obtained directly after the larvæ were treated are recorded under " Period I " in the tables. A second group of determinations, made six hours after treatment, is recorded under Period II. Larvæ removed from the flasks directly and those which had been in the manometer were kept in individual cages to see if they recovered. Only those measurements obtained from larvæ able to resume normal movement and feeding and finally to pupate appear in the tables. Thus respiration of paralyzed larvæ but not of dying ones was measured.

Oxygen consumption was measured at seven different temperatures namely 10° C., 15° C., 20° C., 25° C., 27° C., 30° C., and 35° C. Manometers were placed in water baths which controlled temperatures to ± 0.5° C.

Determinations were repeated until results from three larvæ, all of which recovered from the anesthetic, were obtained. Oxygen consumption of three individual larvæ paralyzed by parasitic sting was measured. Determinations on the oxygen consumption of three normal larvæ, untreated with anesthetics and not exposed to the attacks of the parasite were used as control. Carbon dioxide production was determined for each of the larvæ used and the respiratory quotients calculated. Results in the tables are averages of three determinations each. The temperature coefficient or Q_{10} was calculated between the intervals of temperature at which determinations were made.

RESULTS

Oxygen consumption of normal untreated larvæ increased directly with the temperature until 32° C., at which point it decreased sharply. The respiratory quotient, however, showed little or no change until the temperature rose to 32° C. Between 30° C. and 32° C. the respiratory quotient dropped from 0.91 to 0.83 (see Table I). Since the full life cycle of the Mediterranean flour-moth cannot be completed at temperatures much above 32° C., it would appear that the lowering of the respiratory quotient into a range indicating protein oxidation indicates some degree of injury.

TABLE I

Comparative data on oxygen consumption of normal full-grown Ephestia larvæ and Ephestia larvæ stung by Microbracon hebetor

Temperature in degrees Centigrade	Oxygen consumption in cc. per gram per hour		Q_{10}		Respiratory quotient	
	Normal larvæ	Stung larvæ	Normal larvæ	Stung larvæ	Normal larvæ	Stung larvæ
10	24.2	18.6			0.91	0.92
15	36.4	18.2	2.18	1.00	0.92	0.89
20	42.0	21.	1.73	1.12	0.89	0.91
25	53.6	25.3	*1.47 (2.54)	*1.32 (2.04)	0.92	0.88
27	61.5	27.5	†1.04	†1.72	0.93	0.93
30	72.3	31.	1.72	1.476	0.91	0.87
32	54.7	26.3			0.83	0.81

* Between 15° C. and 25° C. ( ) Between 20° C. and 25° C.
† Between 20° C. and 27° C.

Larvæ treated with ether were not only motionless but appeared soft and flaccid. The relaxation produced by the ether was apparently reflected by the lowered oxygen consumption of the etherized larvæ compared with the normal. As the larvæ recovered they used more oxygen. At all temperatures their oxygen consumption was less than that of untreated larvæ, but at low temperatures this difference was not as marked as at high. Etherized larvæ showed a similar decrease in oxygen consumption between 30° C. and 32° C. to that of untreated larvæ (see Table II). After six hours oxygen consumption had returned nearly to normal levels. After twenty-four hours etherized larvæ could not be distinguished from untreated by either their appearance or by their oxygen consumption. Etherized larvæ from which the records were made were able to spin cocoons and to emerge as normal adults.

Directly after treatment with orthodichlorbenzene, larvæ of *Ephestia* were tense and appeared to be in a state of rigor. They held their heads stiffly upright in a sphinx-like position from a tense motionless body. Even six hours after treatment the larvæ appeared somewhat stiffer than normal. In contrast to the larvæ treated with ether, those treated with orthodichlorbenzene showed a greater oxygen consumption than did normal larvæ, over the temperature range from 10° C. to 30° C. inclusive. (Compare Table I, Column 2 and Table II, Column 2.) At 32° C. the oxygen consumption of larvæ treated with orthodichlorbenzene was less than that of the untreated. This greater decrease in oxygen consumption may indicate that larvæ exposed to orthodichlorbenzene are more susceptible to injury by high temperatures than are normal larvæ.

Table II

Oxygen consumption of full-grown Ephestia larvæ treated with orthodichlorbenzene (1 mgm. per liter), and with ether (.568 gm. per liter)

Temperature in degrees Centigrade	Oxygen consumption in cc. per gm. per hr.				Q_{10}			
	Period I		Period II		Period I		Period II	
	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$
10	22.3	24.3	23.4	23.2				
15	33.5	39.3	35.7	37.1	2.99	2.12	3.04	3.2
20	37.4	47.1	44.1	43.5	1.677	1.98	1.84	1.87
25	42.7	58.5	52.3	52.7	*1.304 (2.28)	*1.49 (2.48)	†1.46 (2.39)	1.42 (2.46)
27	45.6	67.4	61.	60.9	1.95	†2.04	†1.51	2.0
30	52.1	80.5	69.9	73.1	1.39	1.73	1.58	1.68
32	45.8	46.7	51.6	53.8				

* Between 15° C. and 25° C. ( ) Between 20° C. and 25° C.
† Between 20° C. and 27° C.

Since larvæ exposed to fatal doses of orthodichlorbenzene showed a gradual decrease in oxygen consumption during the period they were in the manometer, they could be distinguished from larvæ exposed to a non-fatal dose. A marked decrease in oxygen consumption during the time that larvæ were in the manometer was interpreted as a measurement of a death process rather than of the specific effect of orthodichlorbenzene.

The respiratory quotient of larvæ treated with orthodichlorbenzene did not differ significantly from that of normal larvæ. In both cases the respiratory quotient dropped from the carbohydrate to the protein range at 32° C. Both normal larvæ and those treated with orthodichlorbenzene showed a marked decrease in oxygen consumption at the same point as that at which the respiratory quotient decreased.

Larvæ stung by *Microbracon* closely resembled those treated with ether. In both cases the larvæ were soft, relaxed and motionless. They consumed less oxygen than either normal larvæ or etherized larvæ throughout the range of temperature used. As measured by oxygen consumption, the sensitivity to temperature change of stung larvæ was far less than for normal larvæ. Thus between 10° C. and 15° C. there was no increase in oxygen consumption by the stung larvæ. At 30° C. the oxygen consumption of the stung larvæ was about that of normal larvæ at 15° C. (see Table I). At 32° C. the oxygen consumption of the stung larvæ decreased from what it had been at 30° C. Also at 32° C. the respiratory quotient showed a marked decrease.

The level of oxygen consumption of the stung larvæ remained approximately constant for at least a week after they were stung since measurements taken a week after their exposure to *Microbracon* were nearly the same as those taken directly after their paralysis. The magnitude of change produced by the *Microbracon* was far greater than that caused by either of the chemical agents used. In the experiments reported here, both ether and orthodichlorbenzene produced a reversible change while the sting of *Microbracon* produced an irreversible change. If the dosage of ether or of orthodichlorbenzene be increased to the point of irreversible change the larvæ die rather than remain quiescent as they do when stung by *Microbracon.*

A few experiments were tried to see if either orthodichlorbenzene or ether would affect larvæ stung by *Microbracon.* As far as could be detected from measurements on respiratory rate and quotient neither orthodichlorbenzene nor ether had any effect on larvæ stung by *Microbracon.* Furthermore, the stung larvæ treated with orthodichlorbenzene did not stiffen nor those treated with ether become more flaccid. Apparently the *Microbracon* sting had already destroyed or damaged the mechanism on which these drugs could act.

Discussion

Levels of respiratory metabolism of *Ephestia* larvæ stung by *Microbracon* are so low and so insensitive to temperature change as to suggest those of cells in diapause such as the " blocked " embryonic grasshopper cells reported by Bodine (1934). The resting condition of the *Ephestia* larvæ differed, however, from that of the embryonic grasshopper cells in that the rest of the grasshopper embryos could be broken with greater ease than could that of the larvæ stung by *Microbracon.* The author has never observed an Ephestia larva recover from *Microbracon* sting. Mr. H. C. Donohoe, in an unpublished communication, informed the author that he had seen *Ephestia* larvæ recover from the sting of the parasite but that recovery was very rare.

Although temperature rise from 10° C. to 15° C. did not change the respiratory rate of *Ephestia* larvæ stung by *Microbracon* and temperature change from 15° C. to 30° C. increased the rate in stung larvæ only one-third as compared to twice in normal larvæ, still temperature rise from 30° C. to 32° C. was associated with a drop in respiratory rate for the larvæ stung by *Microbracon,* as it was so associated with normal larvæ. Thus the stung larvæ, while insensitive to changes in temperature through the range through which normal development occurs, are susceptible to injury from high temperature.

Throughout the temperature range used in the experiments with *Ephestia* larvæ, the respiratory quotient of the larvæ stung by *Microbracon* did not differ from that of normal larvæ. Both were in the carbohydrate range from 10° C. to 30° C.; both dropped to the protein range at 32° C. In so far as the respiratory quotient indicates the type of metabolism, a lack of difference between the respiratory quotient of stung larvæ and of normal larvæ would indicate the same type of metabolism in both. Differences not measured by the respiratory quotient might well occur.

Neither the effect of ether nor that of orthodichlorbenzene duplicated that of the *Microbracon* sting. Ether did produce flaccidity in the larvæ and did lower the respiratory rate. It was impossible to obtain as low a rate with ether as the *Microbracon* sting produces, without killing the larvæ. Orthodichlorbenzene produced a rigidity in the larvæ and also increased respiratory metabolism. Neither chemical agent affected the respiratory quotient.

Hartzell (1935) found that lesions produced by the killer wasp, *Specius speciosus* Dru., in the nervous system of the cicada, *Tibicen pruinosa* Say, were similar to those found in insects killed with triorthocresyl phosphate and the pyrethrins. Injections of formic acid and of acetic acid in meal worms (*Tenebrio molitor* L.), however, failed to produce the lesions characteristic of paralysis.

The finding of Hartzell (1935) of lesions sufficient to be demonstrated microscopically in the nervous system of a cicada stung by a wasp may be correlated with the profound differences in the muscular tonus between normal larvæ and stung larvæ. In turn these alterations of the nervous system associated with marked changes in respiratory rate may point to an intimate connection of the nervous system with respiration.

SUMMARY

1. Larvæ of *Ephestia kühniella* Zeller, when stung by the parasite *Microbracon hebetor* Say, become soft, flaccid and motionless.

2. Associated with the flaccidity of the stung larvae is a greatly lowered respiratory rate through the temperature range used, namely 10° C. to 32° C.

3. Not only does the *Microbracon* sting lower the respiratory rate but it decreases the sensitivity of *Ephestia* larvæ to change in temperature to such an extent that between all temperature intervals used the Q_{10} of larvæ stung by *Microbracon* was less than that of normal larvæ. Between 10° C. and 15° C., the Q_{10} was 1.

4. Orthodichlorbenzene in a median paralytic dosage (just sufficient to cause a reversible paralysis in half the larvæ) produced stiffness and rigidity in *Ephestia* larvæ. Associated with this stiffness was a marked increase in respiration rate beyond that of untreated larvæ except at 32° C. where it fell below that of normal larvæ.

5. Ether in a median paralytic dosage produced flaccidity in *Ephestia* larvæ. Associated with this flaccidity was a marked decrease in respiratory rate, but at no temperature was the decrease produced by ether of the same magnitude as that produced by the *Microbracon* sting.

6. Unlike the respiratory rate, the respiratory quotient was not affected by the parasite sting nor by either of the two chemical agents used.

7. Rise in temperature to the point at which the rate of development of *Ephestia* decreases, namely 32° C., was associated with a drop in respiratory rate and a decrease in respiratory quotient from the range indicating carbohydrate metabolism to that indicating protein. Not only normal larvæ, but also those treated with ether or orthodichlorbenzene or stung by the parasite, showed a decrease both in rate and quotient at 32° C.

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Oxygen consumption and respiratory quotient of the larvæ were determined at the Zoölogical Laboratory of the University of Pennsylvania during the years 1931–1933. Calculations and redeterminations of some of the results were made at the Division of Entomology, University of Minnesota. Thanks are due to Dr. C. E. McClung for the use of laboratory facilities at the University of Pennsylvania and to Dr. William A. Riley for the same courtesy at Minnesota.

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AUTOTOMY IN THE BRACHYURAN, UCA PUGNAX

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A study of the extent to which the anomuran *Porcellana platycheles* will autotomize its legs on single stimulation of successive appendages has shown (Hoadley, 1934) that there is a clear-cut and fundamental difference between the behavior of males and of females bearing eggs. This form is ideal for such experiments in that gently seizing the leg between the points of blunt forceps will ordinarily be followed by autotomy. Males tested in the above fashion autotomized an average of 5.57 + legs per animal (69.6 per cent), the distribution of the autotomies being 4 for the first four trials and 1.57 + for the second four. Quite in contrast to this, the gravid females cast an average of two legs in the eight trials (25 per cent), the distribution being 1.5 and 0.5 in the first and second four trials respectively. Unfortunately but one female not bearing eggs was available at the time of the examination of this material, but a test of that animal showed five legs autotomized (62.5 per cent) (distribution 4 and 1), a figure which compares more favorably with the condition encountered in the male than with that of the remaining females. The suggestion was made, therefore, that the functional state of the animal bearing eggs was different from that of the male and that of the non-gravid female and that this difference was directly reflected in the extent of the autotomization. The present report is based on an examination of autotomy by males, egg-bearing females, and females not carrying eggs, in the brachyuran *Uca pugnax*.

The occurrence of autotomy in the decapod Crustacea and the methods by which this is accomplished have been examined in detail by Wood and Wood (1932). That of the fiddler crab, *Uca pugnax,* has been found by them to be a true autotomy in contrast to autospasy and autotilly, which may be of great importance in other forms. When the autotomy of *Uca* is compared with that of *Porcellana* described above, however, it is at once evident that while *Porcellana* casts its legs on the slightest provocation and hence care in handling the animal is essential, *Uca* casts its legs only after injury so that a relatively harsh treatment is necessary to induce the reflex.

The animals used in the following experiments were obtained from the shore of Rhode Island[1] and brought immediately to the laboratory where the experiments were performed. Two lots of animals were worked with, one obtained in August of 1934 when the females were not bearing eggs and the other in June of 1935 when over half of the females were carrying eggs attached to the abdomen. All of the animals used in the experiments were mature. It was necessary in some of the experiments to use a few animals which had already cast one of the legs but in no case was an animal with more than one leg lacking employed.

Autotomy was induced by injuries of two sorts. In the first a limb was grasped and crushed between the jaws of a pair of plier forceps with a quick motion in order that the leg should actually be held as short a time as possible. Such injuries were generally made in the carpus though the exact position on the segment varied slightly and in several instances the injury was in the distal portion of the meros. It was soon found that holding the animal either by the body or by the leg tested facilitated the autotomy of the member so that the animal was not held during the infliction of the injury. That fact, together with the effort to accomplish the injury in as short a time as possible, led to slight variations in the actual extent of the trauma. In the male, the large claw of the cheliped was crushed across the middle at the base of the dactylus and consequently the injury there was relatively great.

The second type of injury inflicted to induce autotomy was cutting. Legs were cut with a heavy pair of scissors at or near the joint between meros and carpus, removing the carpus and propus. The claw of the cheliped of the male had to be cut by means of a small pair of bone forceps, the cut being located in the same region as the injury by crushing mentioned above. Both types of experiment were carried out in clean moist crystallizing dishes; in addition, crushing experiments were performed in crystallizing dishes, the bottoms of which were covered by filter paper. Only females bearing eggs and males were used in the cutting experiments.

The results of the experiments have been summarized in Table I. The letters following the year indicate the order in which the experiments for that year were done. While every effort was made to perform the experiments in the same way, it is but natural that those done in the same year should be most similar and that those done successively in any one year should be most nearly alike in execution. Thus, of the experiments in which appendages were crushed on glass, 34A (♂'s) and 34B (♀'s) are comparable, as are also 35A (♀'s with eggs) and 35B (♂'s) on the one hand, and 35G (♀'s with eggs), 35H (♀'s without eggs) and 35I (♂'s) on the other. It is evident when these are compared that the stimulation in 35A and 35B was not as effective as in 35G, 35H and 35I. This will be dealt with in some detail below. The results themselves seem to be comparable, however, for when the number of legs autotomized by the males is compared with the number autotomized by the female bearing eggs it is found to be very similar in the two cases being expressed by the ratios 1.60 : 1 and 1.62 : 1 respectively. When the average total number of legs autotomized by the three classes of animals is examined, it is found that, with one exception, the largest number is cast by the males; the females without eggs come next; the females with eggs cast the smallest number. It will also be noted that the females without eggs cast a relatively smaller number of legs in August

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[1] I wish to thank Dr. F. C. Chace for the *Uca* which he was so kind as to collect for me.

Table I

Method	Sex	First 5 trials	Second 5 trials	Total	First 5 trials / Second 5 trials	Year and series	Number of animals	Average first 5 trials	Average second 5 trials	Average total		
Crushing on glass	♂	41	23	64	1.81−	34A	9	4.56−	2.56−	7.1+	♂/♀ = 1.22+	Averages— ♂'s = 7.1 ♀'s = 6.65 ♀ eggs = 4.4
	♀	37	21	58	1.76+	34B	10	3.7	2.1	5.8		
	♀ with eggs	15	5	20	3.0	35A	5	3	1	4.0	♂/♀ eggs = 1.60	
	♂	19	13	32	1.46	35B	5	3.8	2.6	6.4		
	♀ with eggs	16	8	24	2.0	35G	5	3.2	1.6	4.8	♂/♀ = 1.04 ♂/♀ eggs = 1.62	
	♀	16	14	30	1.14+	35H	4	4.0	3.5	7.5		
	♂	23	16	39	1.44−	35I	5	4.6	3.2	7.8		
Crushing on filter paper	♂	21	15	36	1.40	34C	5	4.2	3.0	7.2	♂/♀ = 0.94	
	♀	20	17	37	1.18−	34D	5	4.0	3.4	7.4		
	♀ with eggs	15	9	24	1.67	35E	5	3.0	1.8	4.8	♂/♀ = 1.16† N.B. anterior appendages first in every case	
	♂	17	11	38	1.54+	35F*	5	3.4	2.2	5.6		
Cutting on glass	♂	11	5	16	2.2	34E	5	2.2	1.0	3.2		
	♀ with eggs	11	1	12	11.0	35C	5	2.2	.2	2.4	♂/♀ eggs = 1.5	
	♂	12	6	18	2.0	35D	5	2.4	1.2	3.6		

Average
♂/♀ = 1.067−
♂/♀ Eggs = 1.57+

* The large claw was injured first in each of the experiments of this series; see discussion in text.

† Omitted in the average.

than they did in June. Compared with the males, the index for such animals is 1.22 in August as compared with 1.04 in June, the behavior at the latter time being similar to that of the males. It appears quite evident from the tabulated data that the female bearing eggs behaves quite differently in autotomizing its legs from the male and from the female not bearing eggs. It is also evident that the greater difference is to be found in the second five trials. This can be appreciated most easily by examination of the column in which the relation between the number of legs lost in the first five trials is compared with the number lost in the second. If the number lost in each series is approximately the same the ratio will approach 1 while if the number diminishes materially it will rise. In all of the first group the higher quotients are to be found in animals which are females and carry eggs.

The second type of experiment differed from the first only in that the bottom of the dish in which the animals were placed during the experiment was covered with filter paper. Examination of the totals would appear to indicate that the behavior in this case is similar to that in the preceding. The exception is found in the case of the males of 35F which showed an average total of 5.6 which is well below that of the previous series and also well below those of the males and of the females without eggs used in 34C and 34D. Reference to the protocol of the experiment shows, however, that in this series (35F) the large claw was the first to be injured and this has evidently had a marked effect on the subsequent behavior of the individuals. This might be explained either on the basis of the extent of injury or on the basis of some aid in autotomy rendered by the large claw. While the large claw is used directly to rid the animal of the injured member at times, this is by no means usual and could not, I believe, account for the discrepancy in the results. It is also of interest to note that when the ratio between the autotomies in the first five trials and the second five is examined in this entire group it is found that the number of autotomies is relatively high in the second five and that hence the index is lowered. Again, this is not true of the males in 35F. While no quantitative data are available on this point it should be mentioned that on the filter paper, the amount of bleeding which takes place is greater than when the animal is in the clean glass dish. The possible significance of this will be discussed below, more particularly in relation to the cutting experiments. As in the previous group of experiments, the relation between the total number of autotomies by males and by females not carrying eggs is approximately the same.

Only three sets of experiments were made cutting the appendages of the animals upon glass. Two of these were done in 1935 and one in

1934. Comparing the males of 34E with those of 35D it is found that in both cases the number of legs autotomized is very low, being (as averages) 3.2 in the first instance and 3.6 in the second. These figures are very similar and are characteristic of the individuals of each series of experiments. The females bearing eggs showed a similar low average of 2.4. The immediate result of the experimental procedure differs from that recorded above in that there is far more bleeding after cutting than after the crushing previously discussed. When the males of the 1935 series are compared with the females it is found that the relation approaches that of the crushing experiments, being 1.5. When the number of autotomies in the first five trials is compared with the number in the second five, it is found that for the males the quotient is relatively high, being 2.2 and 2.0; but this is low compared with that for the females bearing eggs, which is 11.0, the highest encountered in any of the experiments. The relation between the males of the two years is 1.11, which is close agreement.

The data obtained in the experiments may be dealt with in two ways. It is possible to compare the total number of legs cast by each group according to the animals composing it, or the number of legs cast in the first five trials may be compared with the number cast in the second five and the quotients examined. The results of each method yield information which is interesting and hence will be considered separately.

The relation between the total number of legs autotomized by comparable groups of animals will be considered first. The most casual examination of the table will show that the males and the females without eggs consistently cast more appendages than did the gravid females. The difference is so great that when the males are compared with females bearing eggs which were tested at the same time the ratio is as 1.57 + (average) is to 1. For the reasons stated in the table the results of tests 35E and 35F have been omitted from this average. It is of especial interest to note that while the method employed may have a great effect on the totals obtained it has not modified the relation between the totals (see "cutting on glass").

When the autotomies of females not bearing eggs are compared with those of the males it is found that in the 1935 tests (June) the results for the two sexes are essentially the same. In the tests of the 1934 series it might at first be thought that the averages in 34A and 34B showed a difference in behavior. A comparison with the results of 34C and 34D which are also comparable leads to the conclusion that the discrepancy in the former case is the result of unequal stimulation rather than of a difference in the animals. This is not unlikely for 34A and 34B were the first *Uca* to be tested. When the results obtained in all of the tests are averaged it is found that the relation between autotomy by the male and by the non-gravid female is as 1.067 — is to 1, the difference not being significant. The variation in results between more widely separated tests of the same year and between tests of different years is to be explained on the basis of manipulation.

A comparison of the number of autotomies in the first five trials with the number in the second five should prove most interesting and should yield important information, for by this method groups of stimulations of individuals are dealt with and these represent tests which are most similar in every respect. Several general statements may be made based on the results of this comparison. It may be stated definitely that in comparable experiments the relative number of autotomies in the first five trials in females bearing eggs is consistently higher than in the remaining females or in the males. On the other hand, the differences encountered in this ratio between animals of this second class (34A and B; 35B, H, and I) of the first group alone is fairly large as is the difference between the gravid females (35A and G). While both the latter values are above those of the first class, the difference between the highest value of the first class and the lower value of the second is relatively small. The ratio in itself becomes more significant after the two components are examined. Insofar as the first five trials are concerned the results are much the same whether the crushing be on filter paper or on glass; as for the second five, the average on the filter paper is in all cases high. This would lower the value. The reverse is particularly marked in the cutting experiments where in males the average of the first five trials is approximately half that in the crushing experiments while that of the second five is nearer one-third. The females with eggs behaved much as the males in the first five trials but in the second five autotomy was practically suppressed so that the relation between the two rises to a value of 11.0. In this connection it should be stated that the results of a few experiments on cutting on filter paper indicate that in that case also there is a similar reduction in the number of autotomies in males. In 35F we find that both the first and the second set have been reduced, the second more than the first. This results in a value for first/second of 1.54 + which approaches that of the female bearing eggs as did the total number of autotomies (5.6 av.).

It has already been mentioned above that relatively harsh treatment is necessary to induce autotomy in *Uca*. Sensory receptors associated with hairs are present in the cuticle covering the limbs. The tactile stimulation of the appendage of *Porcellana* is sufficient to induce the casting of a leg but in *Uca* the actual injury of the member is essential. This, in turn, must result in much more violent stimulation of afferent sensory nerves. As may be seen in the data presented above, the degree of stimulation in different experiments differs and with this variation there is an accompanying variation in the number of autotomies. Evidence of the same sort may be derived from the comparison of cutting experiments and crushing experiments. In the former the number of autotomies is far lower than in the latter. Similarly it might be expected that the extent of stimulation both in degree and in duration would be greater in the case of crushing than in the case of cutting. This probably does not account completely for the discrepancy in the two cases as the amount of bleeding observed differs.

The blood of the Crustacea coagulates very readily. Ordinarily there is relatively little loss of blood following traumatic injury to the limbs. It has been stated above that there is a greater loss of fluid when the appendage is crushed on filter paper than when crushed on moist glass. The loss is far greater when the appendage is cut at or near the base of the carpus. With successive stimulation the latter type of injury eventually results in the loss of considerably more blood than does the crushing. This may contribute to the difference in the behavior of the two types of material.[2] Similarly the extent of the injury to the large claw on crushing must result in the loss of more of the body fluid than would injury to other appendages. This may in part account for the reduction in the total number of autotomies in series 35F. The amount of bleeding following autotomy, i.e. at the breaking joint, is relatively small so that the act in itself tended to restrict the loss of body fluid by the injured animals in the majority of the crushing experiments.

The autotomy reflex of the Crustacea as generally conceived involves the transmission of stimuli along the sensory neurones and transmission of motor impulses by the motor neurone to at least the autotomizing muscle. The path is presumably completed centrally in the ventral ganglion. There are very few motor nerve fibers in the segmental nerves of the crab, indicating that whole muscles and possibly even different muscles may be innervated by collaterals of a single neurone. In the crab the fifth to the thirteenth lateral nerves all have their origin in a common ventral ganglionic mass, the ninth to the thirteenth innervating the five appendages as well as the muscles of the body wall of these segments. In the experiments reported here as well as those on *Porcellana* referred to above, the reflexes which are activated by successive stimulations have a cumulative effect and lead to an inhibition and eventual failure of the response even when the stimulations involve receptors situated in different appendages. In the male of *Porcellana* the inhibition first became evident after the fifth successive appendage was stimulated; in the male of *Uca* the inhibition is usually noted after the fourth stimulation. In females bearing eggs, inhibition appears between the first and the second trials in *Porcellana,* and after the third in *Uca.* Females not bearing eggs appear to behave as males in *Uca* and the indications are that the same is true for *Porcellana.* That there is actually an inhibition and that it is gradually built up is demonstrated by the fact that without exception the number of autotomies in the first five trials is greatly in excess of those in the second five. It is also apparent that the inhibition becomes effective more rapidly in the female bearing eggs than in the male for the relation between the number of legs cast in the first five trials and in the second five yields a higher quotient in the first case. These facts suggest strongly the formation of a block most easily explained in terms of a chemical inhibitor. This inhibition may be effected in the periferal mechanism or in the fused central ganglionic region. The fact that bleeding tends to reduce the number of autotomies also leads us to the conclusion that the inhibitor is of a chemical nature and that it is readily eliminated through the blood, accumulating more rapidly when the amount of blood in the body is reduced.

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[2] Dr. John H. Welsh informs me that some collectors bleed crabs in order to prevent their casting appendages when put into preservatives. He himself has used this method successfully.

One additional observation deserves mention in that it, too, suggests a mutual effect, though in this instance the effect is in terms of stimulation rather than of inhibition. In several instances a delayed autotomy has been observed which took place on the stimulation of another appendage. This is well illustrated by a case in which the third leg stimulated failed to be cast at the time but gave a second response which was successful when the fifth successive leg was stimulated. Both reflexes were typical and the leg was eventually autotomized in the usual fashion. Apparently the nerve impulses in the neighboring units were responsible for the autotomy, possibly through the production of diffusable humoral agents which, in turn, effect the activation of previously stimulated components.

In conclusion it should be emphasized that the evidence for the existence of inhibiting and stimulating substances is indirect and that they have not been demonstrated. However, the behavior of both *Porcellana* and *Uca* may be most readily understood on some such basis. The method of performing the experiment has a definite influence on the kind of result obtained. Not only should a study be made of the components of the blood, but, recognizing the presence of the fused ventral central ganglia, similar experiments should be performed on the crayfish or some like form in which the ganglionic enlargements from which the walking legs are innervated are segmentally arranged and discrete, and where, therefore, mutual influence in the central mechanism would be less likely. The experiments do demonstrate very clearly that, in respect to the extent to which legs are autotomized, there is a fundamental difference between females bearing eggs, on the one hand, and females without eggs and males on the other.

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POLAR BODY EXTRUSION AND CLEAVAGE IN ARTIFICIALLY ACTIVATED EGGS OF URECHIS CAUPO

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INTRODUCTION

In recent articles, Hiraiwa and Kawamura (1935, 1936) report some parthenogenesis experiments on eggs of *Urechis unicinctus*. As their results differ in some respects from those of Tyler (1931*a, b,* 1932*a, b*) on *Urechis caupo* and as one of us (B.) intended to continue the unfinished studies of Bělař on the cytology of parthenogenesis, we decided to test the methods of the Japanese authors on the Californian *Urechis.* The principal point of the mentioned difference is that, in the experiments of Hiraiwa and Kawamura, high percentages of cleavage were obtained in batches of eggs most of which had extruded polar bodies, whereas, in the experiments of Tyler, eggs that extruded polar bodies almost invariably failed to divide. Hiraiwa and Kawamura used heat, hypo- and hypertonic sea water, KCN sea water, and, most successfully, ammoniacal sea water, for which method alone they present the data on polar body extrusion and cleavage. Tyler had used only anisotonic sea water.[2]

It was considered advisable to isolate the various types of eggs according to their behavior in the maturation divisions, a precaution which Hiraiwa and Kawamura failed to take. This becomes of especial importance in view of the fact that after certain treatments and at certain stages blisters may appear on the surface of the egg that may easily be mistaken for polar bodies. The Japanese authors do not mention these blisters though they show them in their drawings. Also it is possible, as was pointed out by Hiraiwa and Kawamura, that when two polar bodies are present on the egg, they may represent the first and second polar bodies or the divided first polar body or two "second" polar bodies. Isolation of the eggs helps to decide these points.

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[1] Fellow of the Rockefeller Foundation.

[2] The Japanese authors erroneously attribute to Tyler the view that cleavage cannot occur in eggs which have extruded both polar bodies. Although it was found that such eggs failed to divide, the possibility was admitted that "by means of other agents or by additional treatment the eggs that extrude two polar bodies may be made to develop" (Tyler, 1931*a*, p. 209).

It may be stated at the outset that the conclusion of Hiraiwa and Kawamura could be confirmed that, after certain treatments with ammoniacal sea water, eggs divide after polar body formation. However, certain reservations must be made, since only low percentages of cleavage were obtained, and also the same exposure that gives cleavage generally produces abnormalities in the maturation divisions, so that it is not certain that the divided eggs with two polar bodies are haploid. This and related points will be cleared up in the cytological investigation. Where one or both maturation divisions are submerged, we find that cleavage almost invariably follows and the type of cleavage is related to the polar bodies produced.

Hiraiwa and Kawamura also report that, by means of a second treatment with ammoniacal sea water applied after the extrusion of the second polar body, eggs which ordinarily would not do so, may be made to divide. Repetition of this experiment on *Urechis caupo* failed to confirm the result. They report too that the "poorly activated" eggs described by Tyler did not appear in their experiments. Using the ammoniacal sea water treatment with *U. caupo,* however, we find again this type of egg after certain treatments, and its behavior is the same as in the hypotonic sea water experiments.

A new agent was also tried in the experiments reported here, namely ammoniacal dilute sea water which gave high percentages of cleavage, without polar body formation. The kind of cleavage, however, differs in an interesting manner from that obtained by the ammoniacal sea water treatment. Acidified sea water was also used in some experiments with results that were essentially the same as in the hypotonic sea-water experiments.

Treatment with Ammoniacal Sea Water

Urechis eggs may be activated by sea water containing ammonia in a wide range of concentrations. Solutions ranging from 0.02 molar to 0.002 molar NH_3 in sea water were employed. The minimum exposure necessary to obtain 100 per cent activation is, of course, shorter the more concentrated the solution. A typical run is presented in Table I. The solution employed in this experiment is the same as that used in some of the experiments of Hiraiwa and Kawamura, and the activating exposures are roughly the same as in their experiments. At other concentrations the results with *Urechis caupo* also approximate those with *U. unicinctus* when allowance is made for the temperature differences.

Also the exposures giving cleavage are in part similar in both cases. But certain differences are evident. In the *U. unicinctus* experiments no cleavage was obtained after short exposures to the activating solution, whereas we obtain cleavage after exposures too short to activate all of the eggs. The eggs that divide after the short treatment are all of the " poorly activated " type previously described and figured (Tyler, 1931*a*, Figs. 27 to 30). They produce no polar bodies. Hiraiwa and Kawamura state that they could not find any such eggs in *Urechis unicinctus*. After somewhat longer exposures, ranging in the tabulated case from 2 to 7 minutes, 100 per cent activation is obtained but none of the eggs divide. This confirms roughly the cleavage-activation relation obtained with hypotonic sea water (Tyler, 1931*b*).[3] But upon still longer exposures cleavage is obtained, rising to about 90 per cent, and then dropping again to zero while the activation remains 100 per cent. Data on the number of polar bodies extruded are also given in Table I. It is at once apparent that after 2 to 7 minutes exposure, which gives 100 per cent activation but no cleavage, both polar bodies are extruded by all the eggs. After the longer exposures giving increasing percentages of cleavage, the proportion of eggs that extrude two polar bodies decreases. From the figures in the table it would appear that at these exposure times eggs with two polar bodies may divide. However, it must be pointed out that these counts are only approximate, and also that it is especially after such exposures that blisters very much re-

TABLE I

Activation with ammoniacal sea water. Solution = 0.01n NH_3 in sea water, temperature = 20° C.

Length of exposure	Total activation	Poorly activated	Polar bodies			First cleavage		
			0	1	2 or 3	2-cell	3-cell	4-cell
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
¼	40	70	75	5	20	60	3	2
½	90	30	—	—	—	20	0	0
1	100	15	15	0	85	15	1	0
2, 3, 5, and 7	100	0	0	0	100	0	0	0
10	100	0	10	20	70	20	10	2
15	100	0	40	30	30	10	40	45
20	100	0	—	—	—	10	45	25
25	100	0	60	15	25	5	20	15
30	100	0	—	—	—	5	10	10
40	100	0	100	0	0	0	0	0

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[3] See also Dalcq, Pasteels and Brachet (1936).

sembling polar bodies may appear. In Fig. 3 a blistered egg is shown and in Fig. 4 the same egg, ten minutes later, shows the blisters gone and no polar bodies present. Some eggs become covered with blisters so that polar bodies, if present, are completely obscured. The blistering usually appears at some time between extrusion of the second polar body and cleavage, and often reappears during cleavage. In many cases the blisters might easily be mistaken for polar bodies. Preserved eggs would, of course, be better material on which to make the counts, but the isolation experiments reported below give a satisfactory basis for the conclusions.

Cleavage of Eggs Isolated According to Number of Polar Bodies Produced

The eggs that divide after short exposures to the ammoniacal sea water all fail to extrude polar bodies just as in the hypotonic sea water experiments. One hundred such eggs were isolated from a batch treated for 1½ minutes with 0.005 n NH_3 in sea water. All of the eggs divided into two cells at 2 to 2½ hours after treatment. No signs of submerged maturation spindles occurring were noted in the living egg, and it is likely that the first cleavage division is the equivalent of the first maturation division as in the case of eggs "suboptimally" activated with hypotonic sea water (Tyler, 1932*a*).

Table II

Polar body extrusion and cleavage. Eggs treated for 15 minutes with 0.01 n NH_3 in sea water. Isolated 60 to 80 minutes later. Eighty-five to 90 per cent cleavage in main batch.

	Number of eggs	Number cleaved
No polar bodies	47	47
One polar body	12	12
Two polar bodies	74	43

The eggs that divide after the longer exposure behave differently. The first cleavage may give two, three or four cells. There are two types of three-cell cleavage. One type produces three approximately equal-sized cells directly (Fig. 10). The other first attempts to go into four cells, then the cleavage plane between two of the cells disappears giving a three-cell egg in which one of the cells is equal in size to both of the others (Fig. 14). This type should be considered a four-cell cleavage. There are also two types of two-cell cleavage, one of which is derived from an attempted three-cell cleavage. The different types of cleavage correspond to differences in polar body extrusion as will be shown below.

In order to determine the relation between polar body extrusion and cleavage, eggs that were given a treatment with ammoniacal sea water sufficient to induce a high percentage of cleavage were isolated according to whether they produced two, one or no polar bodies. Table II contains data of this sort from four experiments. The eggs were isolated at about 20 to 40 minutes after the time of appearance of the second polar body. It may be seen from the table that all of the eggs with no polar bodies and with one polar body divide and also more than half of those with two polar bodies. However, it is not certain here that the two polar bodies represent the first and second polar bodies. To check this point more carefully the isolation was begun at the time of appearance of the first polar body, eggs with no polar bodies being separated from those with one. Then, at the time of appearance of the second polar body, eggs with two, one, or none were separated from the first group, and eggs with three, two or one from the second. The results of five experiments are summarized in Table III. Of 60 eggs that at first showed no polar bodies, nine later produced one polar body and twelve produced two. Of 134 eggs that at first had one polar body, 43 later showed two and 89 showed three. Actually some of the eggs isolated as having two polar bodies may later have formed three, since this was not carefully checked in each case. However, there are no significant

TABLE III

Polar body extrusion and cleavage. Eggs treated for 15 minutes with 0.01n NH_3 in sea water. Isolated immediately after appearance of first polar body and again at time of appearance of second polar body. Seventy-five to 90 per cent cleavage n main batch.

	Polar bodies produced	Number of eggs	Cleavage at 1½ to 2½ hours			Cleavage at 3½ to 5 hours			
			2-cells	3-cells	4-cells	2-cells	3-cells	4-cells	>4-cells
From eggs with no polar bodies	None	39	4	10	24	0	0	0	38
	1	9	2	6	1	0	1	0	8
	2	12	11	1	0	0	0	1	11
From eggs with one polar body	1	2	1	1	0	0	0	0	2
	2	43	3	2	2	2	1	3	11
	3	89	8	4	0	11	5	3	20

differences in the behavior of these latter two classes.[3] It may be seen from the table that at 1½ to 2½ hours after the start of the treatment in the first group cleavage is obtained in 38 out of the 39 eggs with no polar bodies, in all of the 9 eggs with one polar body and in all of the 12 eggs with two polar bodies. In the second group the two eggs with one polar body both divide at this time, but only 7 out of the 43 with two polar bodies and 12 out of the 89 with three polar bodies. Later, at 3½ to 5 hours, more cleavage is obtained in these last two classes, 17 out of the 43 with two polar bodies and 39 out of the 89 with three polar bodies. Thus, while practically all of the eggs with no polar bodies in the first group divide, only 14 per cent of the eggs with two and three polar bodies in the second group divide at the same time. Considerably later 42 per cent of the latter divide, but it is questionable whether, in most of these, anything like real cleavage had occurred (see below).

The type of cleavage that the eggs undergo is also given in Table III. It may be seen that most of the eggs that extrude no polar bodies divide at once into four cells. The four eggs, listed in the table, that divided into two cells and the ten that went into three cells were all eggs that had attempted to go into four cells. These eggs all show in the living condition, shortly after the time at which the second polar body should appear, four distinct nuclei (Figs. 13 and 15), which quite evidently result from submerged maturation divisions. The nuclei are visible for about 10 minutes, but it is apparent that the four centers for the first division arise from the centers associated with each of these nuclei. These centers evidently do not divide before the first cleavage, since the division is into four, not eight cells.

The eggs that extrude one polar body divide into three cells, the two eggs listed that went into two cells being derived from an attempted three-cell cleavage and the one egg that went into four being an exception. Since this type of egg is isolated from eggs that showed no polar body formation at the time when the first should appear, the single polar body is evidently the second polar body. In the living condition (Figs. 9 and 11) three nuclei are seen shortly after the time of extrusion of the second polar body.

The eggs of this group that extrude two polar bodies divide into two cells, one exception having gone into three. The two polar bodies of these eggs are both seen to arise simultaneously at the time of second polar body extrusion and evidently represent two "second polar" bodies. That is, the first maturation division was submerged, then each of the nuclei produced a polar body at the time of second polar body extrusion. The two polar bodies of such an egg (Fig. 5) are usually further apart than in the case of a first and second polar body (Fig. 2). Two nuclei later appear in these eggs. If their history were not known such eggs might be assumed to have normal first and second polar bodies, or to have simply a divided first polar body.

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[3] In normally fertilized eggs of *Urechis* the first polar body often fails to divide.

In the second group, two of the 134 eggs that had extruded the first polar body failed to extrude the second. These were isolated rather late and are doubtful cases. It appears from this as well as other observations that when the first polar body is extruded, the second almost immediately follows. Those eggs of this group that divided went for the most part into two cells at once. Many of them stop in the two, three or four-cell stage and in most of those listed in the table as having more than four cells, cleavage had occurred in only one of the original cells. Very often, too, it becomes difficult to decide in the living condition whether cleavage or some sort of fragmentation or lobulation had occurred. About two-thirds of the "two and three polar body" eggs that were listed as cleaved at the later time in Table III were actually in the condition illustrated in Fig. 12, showing small lobules. A more detailed study of these eggs would be necessary in order to decide whether cleavage had actually occurred. The number of lobes present is generally considerably greater than the number of cells to be expected at that time.

Plate I

Explanation of Figures

Artificially activated eggs of Urechis; eggs of Figs. 1 and 2 treated for 5 minutes with 0.01n NH_3 in sea water; those of Figs. 3 to 6 and 9 to 16 treated for 15 minutes with the same solution; those of Figs. 7 and 8 treated for 5 minutes with 0.004n NH_3 in 40 per cent sea water.

Fig. 1. Showing first polar body extruded at the normal time.

Fig. 2. Same egg with second polar body.

Fig. 3. Blisters appearing on an egg at the time of polar body appearance.

Fig. 4. Same egg photographed in the same position ten minutes later, showing disappearance of blisters.

Fig. 5. Showing two polar bodies extruded simultaneously at the time when the second polar body should normally appear; the two nuclei present in this type of egg not visible here. The two polar bodies are further apart than in the case of a first and second polar body.

Fig. 6. First cleavage of the same egg shown in Fig. 5.

Fig. 7. "Poorly activated" type of egg.

Fig. 8. First cleavage of the same egg shown in Fig. 7.

Fig. 9. Showing one polar body extruded at the time when the second polar body should normally appear; the three nuclei remaining in the egg are visible.

Fig. 10. First cleavage of the same egg; polar view.

Fig. 11. Another egg showing three nuclei present after the time when the second polar body normally appears; a single polar body is present but not in focus.

Fig. 12. "Lobulation" of an egg in which polar body extrusion was apparently normal; taken when other eggs of the same lot were in 16 to 64 cells.

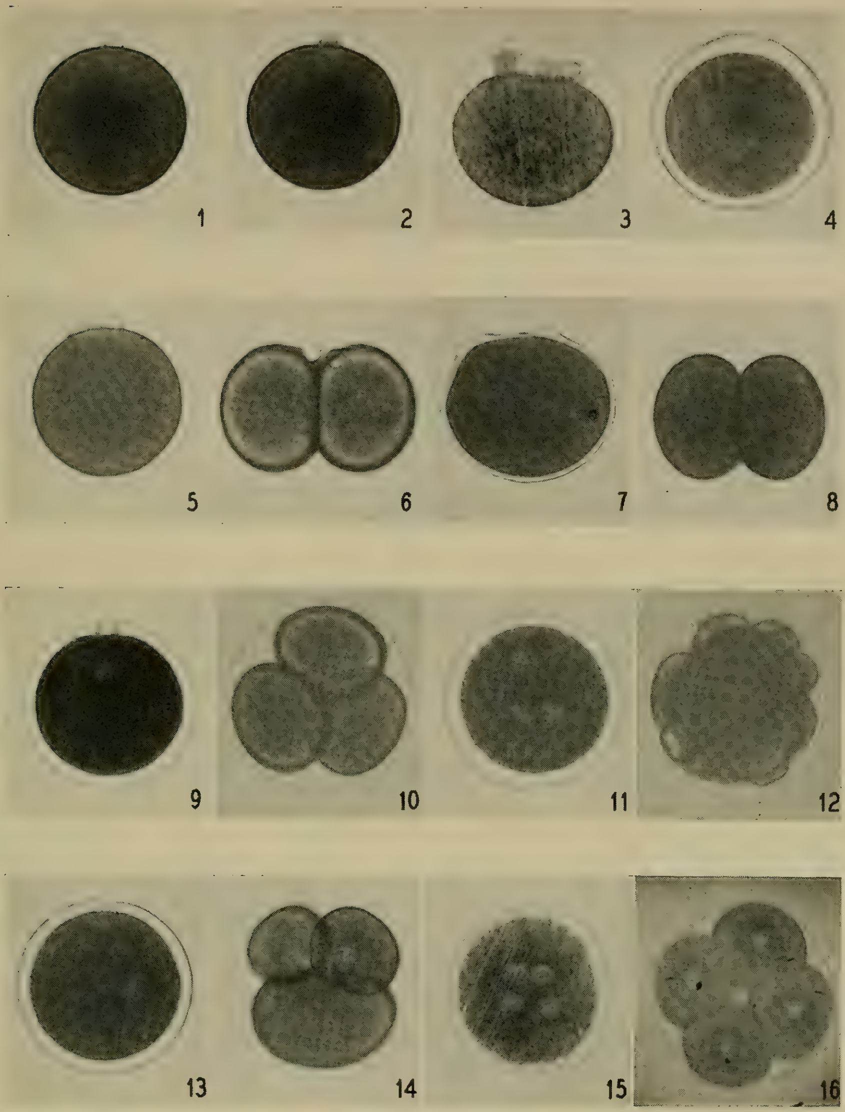

PLATE I

FIG. 13. Egg with four nuclei present after the time when the second polar body normally appears; no polar bodies are present but the pole is indicated by the slight dent in the membrane.

FIG. 14. First cleavage of the same egg, polar view; the three cells result from a disappearance of the cleavage plane between two cells.

FIG. 15. Another egg showing four nuclei present after the time when the second polar body normally appears.

FIG. 16. First cleavage of another egg of this type.

TIME OF POLAR BODY EXTRUSION

The time of appearance of the polar bodies varies with the length of exposure. This is illustrated in Table IV, in which the time of appearance of the first polar body is given. The time from the start of the treatment becomes increasingly greater, the longer the exposure to the activating solution. If the length of exposure is subtracted, we might expect the same value for all lengths of treatment. This, however, is not the case, as the last column in Table IV shows. The time first decreases with increasing length, then rises slightly. We may interpret this to mean that about four to five minutes of developmental progress is made during the sojourn in the solution. Thus, for exposures up to five minutes, the first polar body appears at approximately the same time from the start, and for longer exposures the delay in appearance of the first polar body corresponds to the additional time of treatment. This differs from the results with dilute sea water in which, if allowance is made for the treatment, the time of polar body extrusion is the same for all exposures (Tyler, 1931*a*). However, no developmental changes take place in the hypotonic sea water, whereas in the ammoniacal sea water breakdown of the germinal vesicle and membrane elevation is seen to occur. These changes occur in the solution at about the same rate as in normally fertilized eggs. It appears then that in the case of the longer exposures the observed changes occurring in the solution correspond to considerably more than the four or five minutes necessary to account for the delay in appearance of the first polar body. This means then that while certain changes such as membrane elevation proceed at a normal rate in the solution, other processes leading to polar body extrusion are blocked after four or five minutes. Without elaborating on this, it may be pointed out that a

TABLE IV

Time of extrusion of first polar body. Eggs treated with 0.01n NH_3 in sea water.

Length of Exposure	Time from Start of Treatment	Time from Removal from Solution
minutes	*minutes*	*minutes*
1	34½	33½
2	34	32
3	34	31
5	35	30
7	36	29
10	38½	28½
15	44	29
20	50	30

possible explanation of the increasing percentage of eggs that fail to extrude polar bodies after increasing lengths of exposure may well be the lack of synchrony of the early changes.

It may also be suspected that in those cases in which cleavage is obtained after polar body extrusion the maturation divisions do not proceed normally, but that some of the chromosomes may fail to reach the maturation spindles or that the polar bodies may not receive a full complement. Evidence of this is contained in the work of Hiraiwa and Kawamura, who find often more than the haploid number of chromosomes on the first cleavage amphiaster and also polar bodies devoid of chromosomes. To establish this point, however, it would be necessary to show that no normal maturation divisions occur after treatments giving 100 per cent cleavage, or, where less cleavage is obtained, that a corresponding percentage of abnormal maturation divisions occurs. It may be possible to determine this in the cytological investigation.

Double Treatment

After certain lengths of exposure to ammoniacal sea water 100 per cent activation with normal polar body extrusion is obtained. No cleavage, however, occurs (Table I). Hiraiwa and Kawamura report for *Urechis unicinctus* that if such eggs are given a second treatment with ammoniacal sea water immediately after the extrusion of the second polar body, as much as 37 per cent cleavage may be obtained. We have tried to repeat this but with no success. In Table V three sets of re-treatment experiments are presented. In the first set the second series of exposures is given at various times ranging from 2 to 64 minutes after extrusion of the second polar body. With the exception of the first series of second exposures, less cleavage is obtained than by the single treatment. The slightly higher percentage of cleavage obtained in the series started at two minutes may be significant. The time of extrusion of the second polar body is taken when about 50 per cent have reached that stage. Eggs developing in an unshaken vessel show considerable spread in the time of polar body extrusion, which at 20° C. may be as much as four or five minutes and much more at lower temperatures. It is likely then that in the series begun at two minutes, a number of the eggs had not extruded the second polar body. In the second set in Table V the second treatment is begun at 11 minutes before the extrusion of the second polar body. In this case 5 per cent cleavage is obtained after 15 minutes of second treatment whereas the first treatment gave no cleavage. In the third set hypertonic sea water is used for the second treatment, but no increase in cleavage is obtained.

Three other sets of experiments similar to the first of Table V and using 0.003n, 0.005n and 0.01n NH_3 in sea water for the second treatment, gave similar results.

That cleavage may be obtained after a second treatment applied before the extrusion of the second polar body was shown in the hypotonic sea water experiments (Tyler, 1932*b*). But in those experiments, too, a second treatment applied after polar body extrusion gave no cleavage. Negative results, of course, do not prove very much. It is entirely possible that *U. caupo* and *U. unicinctus* respond differently to identical treatments, and that by varying such factors as the time treatment, the length of exposure, the temperature, the concentration and the kind of activating agent, cleavage might also occur in *U. caupo*. It is also possible that in the experiments of Hiraiwa and Kawamura some of the eggs had not given off the second polar body when the second treatment was begun.

TABLE V

Double treatment. Solution A = 0.005n NH_3 in sea water. Solution B = 0.01n NH_3 in sea water. Solution C = 3.0 g. NaCl in 100 cc. sea water. Temperature 20.1 ± 1° C.

Treatment	Solution	Exposure	Activation	Time after second polar body extrusion of start of treatment	Cleavage
		minutes	*per cent*	*minutes*	*per cent*
First........	A	5	99	—	2
Second......	A	5 to 40*		2	2 to 4
Second......	A	5 to 40*		6	1 to 2
Second......	A	5 to 40*		12	1
Second......	A	5 to 40*		20	1
Second......	A	40		64	1
First........	B	2	100	—	0
Second......	B	1 to 5½		−11	0 to 0.1
Second......	B	10		−11	2
Second......	B	15		−11	5
First........	A	5½	99	—	0.5
Second......	C	2		3	0.5
Second......	C	5 to 40*		3	0.5 to 0
Second......	C	2		15	0.3
Second......	C	5 to 30*		15	0.2 to 0

* Five-minute intervals.

TREATMENT WITH AMMONIACAL-DILUTE SEA WATER

Hypotonic sea water is not always effective in activating *Urechis* eggs. It generally fails to activate eggs that have stood in a dish for more than two hours, or that have been taken from animals kept in the laboratory more than a week. If, however, ammonia is added to the dilute sea water 100 per cent activation and a high percentage of cleavage may be obtained. An experiment with 0.004n NH_3 in 40 per cent sea water is given in Table VI. After five minutes exposure 100 per cent activation is obtained and 90 per cent of the eggs divide. Very few (3 per cent) of the eggs extrude polar bodies and it is apparently only the "no polar body" eggs that divide. The first cleavage, however, is into two cells (Fig. 8). The eggs resemble the "poorly activated" eggs obtained by the short treatment with ammoniacal sea water or the straight dilute sea water treatment. These eggs, too, show rather poor membrane elevation until the time of first cleavage (Fig. 7). With other agents, this type of egg is obtained principally when the total activation is low. Here, however, with treatment giving 100 per cent activation, practically all of the eggs are of that type.

TABLE VI

Ammoniacal hypotonic sea water. Solution = 0.004n NH_3 in 40 per cent sea water. Temperature = 20° C.

Exposure	Activation	Polar bodies			Cleavage		
		0	1	2 or 3	2-cells	3-cells	4-cells
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
3	90	98	1	1	85	0.1	0.1
4	95	95	2	3	95	1	0.5
5	100	97	1	2	90	0.2	0.1

ACTIVATION OF "BLOCKED" EGGS

It has been shown (Tyler and Schultz, 1932) that fertilization may be reversed in *Urechis* by treatment with acidified sea water within three minutes after insemination. The spermatozoa remain in such eggs, but they can be re-inseminated and thereupon they behave as polyspermic eggs. If, instead of re-inseminating, the eggs are given the kind of treatment with dilute sea water by which polar body extrusion but no cleavage is obtained, they develop as normally fertilized eggs. This experiment was repeated using ammoniacal sea water and the results are shown in Table VII. The control eggs (B) are placed in sea water removed from the blocked eggs in order to check the possibility that the ammoniacal sea water merely stimulates the extra sperm present in the dish. The results show that this is not the case, since no cleavage is obtained after the four minutes exposure of the control eggs. From the blocked eggs, however, 80 per cent cleavage and 60 per cent normal development are obtained. With the longer treatment 10 per cent of the control eggs divide and 60 per cent of the blocked ones. The percentage of normal development is lower in this case, indicating some deleterious action of the longer treatment.

These results show that the treatment giving 100 per cent activation and polar body extrusion but no cleavage actually provides sufficient stimulus for development. The ability of the blocked egg to divide is not due to any additional stimulus by the spermatozoön present but rather to the ability of the sperm aster to form an amphiaster.

TABLE VII

Activation of "Blocked" Eggs

Set A. Eggs placed in acid sea water (0.2 cc. of 0.5n HCl in 100 cc. sea water) at 1 minute after insemination. Removed after 20 minutes.

Set B. Unfertilized eggs placed in sea water taken from A. Both sets treated with 0.01n NH_3 in sea water.

Set	Exposure	Activation	Cleavage	Normal embryos
	minutes	*per cent*	*per cent*	*per cent*
A	4	100	80	60
B	4	100	0	0
A	10	100	60	20
B	10	100	10	0

Another kind of experiment shows the non-additive nature of the stimuli provided by the sperm and by artificial activation. If after treatment with dilute sea water or ammoniacal sea water sufficient to induce 100 per cent polar body extrusion but no cleavage, the eggs are transferred to a sperm suspension, fertilization, normal maturation, and normal development occurs. The combined action of the activating agent and the sperm is therefore not equivalent to a prolonged treatment with the activating agent, but simply equivalent to insemination of untreated eggs.

ACTIVATION WITH ACIDIFIED SEA WATER

Lefèvre (1907) activated eggs of *Thalassema* by means of acid and reported obtaining cleavage with or without polar body extrusion.

The solutions employed by Lefèvre were tried on *Urechis* eggs and were found to be strong enough to kill or injure the eggs in one or two minutes. Using weaker solutions good activation could be obtained. In Table VIII an experiment with 0.25 cc. of 1.0n HCl in 100 cc. sea water is presented. This is just about the lowest concentration of acid that gives activation, 0.2 cc. being too weak even after long exposures. It may be seen from the table that the activation rises rapidly with length of exposure and at the same time the percentage of cleavage drops off. It is almost exclusively the " poorly activated " type of egg that divides. These eggs, of course, produce no polar bodies. With other acids and other concentrations the results are similar. If three times the above concentration of acid is used, 100 per cent polar body extrusion with no cleavage is obtained after one-half-minute exposure.

Table VIII

Activation with acidified sea water. Solution = 0.25 cc. of 1.0n HCl in 100 cc. sea water. Temperature = 20° C.

Exposure	Total activation	"Poorly" activated	Polar bodies			Cleavage		
			0	1	2 or 3	2-cells	3-cells	4-cells
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0	—	—	—	—	—	—	—
2	75	95	95	2	3	60	10	0.2
3	100	25	15	15	70	12	0	0
5	100	10	5	10	85	5	0	0
7	100	0	1	4	95	0	0	0
10 to 30	100	0	0	0	100	0	0	0

Discussion

It should be pointed out that we do not consider it impossible to obtain cleavage and normal development after extrusion of both polar bodies in *Urechis*. The point to be made, however, is that when eggs with no polar bodies and with two polar bodies are isolated from the same treated lot, practically all of the former divide whereas very few of the latter show real cleavage. In the " no polar body " eggs obtained by prolonged exposure to ammoniacal sea water, it is clear from the presence of four nuclei that submerged maturation divisions had occurred. In the " no polar body " eggs obtained by short exposure to ammoniacal sea water or to ammoniacal-dilute sea water the behavior is similar to that reported for dilute sea-water activation, no maturation divisions occurring until the first cleavage. The first type divides into four cells at once, the second type into two.

The behavior of the chromosomes during cleavage in the first type of egg has not yet been worked out. In the second type both haploid and diploid cleavages have been found, although the embryos examined show only the diploid number (Tyler, 1932*a*). In a recent article on artificially activated frog's eggs Parmenter (1933) lists among the possible methods of regulation to diploidy this utilization of what is the equivalent of a maturation division spindle for the first cleavage. But it should be noted that if reduction proceeded normally in the first two cleavages only the haploid number of chromosomes would result. Since diploid as well as haploid later cleavages are observed, regulation must occur but this type of behavior certainly does not insure diploidy.

The same treatment that results in both maturation divisions occurring submerged also gives eggs in which only the first spindle is submerged. These eggs may produce either one or two polar bodies at the time of second polar body extrusion, and practically all the eggs cleave. When one polar body is produced three nuclei remain in the egg (visible for a short time) and the first cleavage is into three cells. If two polar bodies are produced two nuclei remain in the egg and the first cleavage is into two cells. Eggs that produce the first polar body almost invariably extrude the second. One should expect this to be the case from the manner of treatment. With the shorter exposures the polar bodies are extruded normally in all the eggs. With longer exposures changes are evidently produced in the egg that prevent polar body extrusion, even when the treatment ends before the time of first polar body formation. If the first polar body appears it simply means that these changes have not been produced or that the egg has recovered before that time. The second polar body should therefore follow. The extrusion of one or two second polar bodies after a submerged first division would mean a recovery of the egg in the interim. On this basis eggs in which the first division is submerged should occur after lengths of treatment that are intermediate between those giving only normal polar body formation and those giving only submergence of both maturation divisions. This is in fact the case.

The eggs that extrude two polar bodies have evidently received an adequate stimulus for development, as the experiments with " blocked " eggs show. That they generally fail to divide in our experiments is probably due to the inability of the centrosome left in the egg after polar body extrusion to divide. Those cases of cleavage after polar body extrusion may then result from division of this centrosome. In the four-cell stage of the " no polar body " eggs where the polar spindles appear to serve for cleavage, the centrosome of one of the cells is equivalent to the one left in the egg after normal polar body extrusion. Yet in this case it continues to divide. The difference may be merely one of position in the cell. Centrifugation, in view of the experiments of Morgan and Tyler (1935), might be of use in examining these questions.

In the dilute sea-water experiments the type of " no polar body " egg with submerged maturation divisions was not obtained. The difference in the reaction of the egg while in the dilute sea water and in the ammoniacal sea water may account for this. With the former agent no developmental changes occur until removal to sea water. With the latter, development starts in the solution. Short exposures to the latter agent give results similar to those by dilute sea water. Prolonged exposures allow the development of the polar spindle to proceed, but either by modifying the surface or in some way preventing the movement of the spindle to the surface do not result in polar body extrusion. This effect is not produced by dilute sea-water activation since no developmental changes occur in the agent. The interest in activation by dilute sea water concerned primarily this fact. With all other parthenogenetic agents, continued exposure after the maximum of activation is reached soon results in injury and death. With dilute sea water in certain concentrations, continued exposure after the maximum of activation results in a falling-off of the activation to zero without injuring the eggs or affecting even their fertilizability.

SUMMARY

1. *Urechis* eggs activated by prolonged exposure to ammoniacal sea water give a high percentage of cleavage as Hiraiwa and Kawamura showed, but such exposures also give a high percentage of polar body suppression.

2. Isolation of eggs of known polar body history shows that practically all those eggs divide that fail to extrude the first or the first and second polar bodies, but only a small percentage of those eggs that extrude the first and second polar body.

3. Eggs that fail to extrude the first polar body may produce two, one or no polar bodies at the time when the second polar body should appear. Later, two, three or four nuclei are visible in the egg corresponding to whether two, one or no polar bodies are present. The first cleavage of these three types gives two, three or four cells respectively.

4. Eggs that extrude the first polar body almost invariably produce the second.

5. Determinations of the time of polar body appearance after various lengths of exposure indicate that not more than four or five minutes progress toward polar body extrusion is made during sojourn in the solution, whereas other developmental changes progress further.

6. Eggs that had been given the kind of first treatment that results in 100 per cent normal polar body formation but no cleavage could not be induced to divide by a second treatment applied at various times after the extrusion of the second polar body. Cleavage was, however, obtained when the second treatment was started before the time of extrusion of the second polar body.

7. The "poorly activated" type of egg that divides without polar body formation or evidence of submerged maturation division is obtained by short exposures to ammoniacal sea water, to ammoniacal-dilute sea water, or to acid sea water, the second agent giving the highest percentages. The first cleavage is into two cells.

8. Normal development is obtained when "blocked" fertilized eggs are activated with the treatment that ordinarily results in 100 per cent polar body formation but no cleavage. Also superposition of fertilization after such treatment results in normal development.

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CROSS-REACTIVITY OF VARIOUS HEMOCYANINS WITH SPECIAL REFERENCE TO THE BLOOD PROTEINS OF THE BLACK WIDOW SPIDER

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It was reported by Graham-Smith in Nuttall's well known book (1904) that an antiserum against the blood of *Limulus* reacted with spider serum, even more strongly than with crab serum, but neither the species of spider nor any further details were given. Since Nuttall's sera were prepared by injecting rabbits with the whole blood serum of the species studied, it remained uncertain if the cross-reaction of *Limulus* and the spider were due to serologically similar blood pigments or to small amounts of some other protein.

I have found that an antiserum prepared by injecting the carefully purified hemocyanin of *Limulus* into rabbits (Hooker and Boyd, 1936) reacted with the diluted serum of the black widow spider. The only other species tested, outside of the injected antigen, which reacted with this antiserum was crab (*Cancer irroratus*), which gave a weaker reaction. The cross-reactions of a number of hemocyanins are set forth in Table I.

It will be noted that no other antiserum reacted definitely with the spider serum, and that the anti-crab serum did not react definitely with the *Limulus* hemocyanin, so that the relationship between these two antisera and antigens is not reciprocal, as is often the case. Of the other hemocyanins, *Cancer* and *Homarus* cross-react to a considerable degree, as do *Vivipara* and *Busycon,* paralleling their relationship.

Absorption of the anti-*Limulus* serum with the optimal (Dean and Webb, 1926) dose of spider serum removed all reactivity to spider but left considerable reactivity to *Limulus;* the limiting titer of the antiserum against *Limulus* remaining about the same. By determination of the optimal proportions ratio, which provides an index of the antibody content (the larger the ratio, the lower the content of antibody), it was possible to show that considerable antibody had been removed (the ratio rose from 780 to 2970). This phenomenon of partial cross-reactivity in related species is familiar (Hooker and Boyd, 1934), and could probably be made the basis of a quantitative measure of serological relationship. The complete removal of all reactivity to one antigen, leaving some to another, presumably indicates a common or closely related antigenic determinant in the two proteins, although in the present case it must be remembered that ultracentrifugal analysis of *Limulus* hemocyanin has revealed three distinct proteins with different molecular weights, and possibly only one or two of these might be concerned in the present cross-reaction. In the case of species as closely related as the hen and duck, absorption of the antiserum for one crystalline egg albumin with the other in most cases hardly affected the optimal ratio against the injected antigen (Hooker and Boyd, 1934) ; a greater effect would have been expected.

Determinations of copper and nitrogen (for methods, see Hooker and Boyd, 1936) on the diluted spider serum showed the presence of 0.522 mg. of N per cc., and 0.0054 mg. Cu per cc. This gives a ratio nitrogen to copper of 96.7, not very different from the ratio of nitrogen to copper of 103 which follows from the values for *Limulus* hemocyanin (Redfield, 1930, Redfield et al., 1928). In some *Limulus* preparations I have found values as low as this or slightly lower. Montgomery (1930) says " In the case of *Limulus,* the hemocyanin appears to account for about 95 per cent of the protein of the serum." It would seem that the above data are consistent with the view that the serum protein of the black widow spider is chiefly or entirely a hemocyanin having about the same copper and nitrogen content as that of *Limulus,* which it closely resembles serologically. According to D'Amour et al. (1936), however, others have reported the presence of hemolysins in the legs of spiders, which presumably means that there may be present in the blood small amounts also of these substances, probably proteins.

TABLE I

Cross-reactivity by the precipitin test of various hemocyanins

Antiserum against purified hemocyanin of:	Antigen: Hemocyanins of:					
	Black widow spider	*Limulus*	*Homarus*	*Cancer*	Snail (*Vivipara*)	*Busycon*
Limulus............	+	++	−	+	−	−
Homarus...........	−	−	++	+	−	−
Cancer.............	?	?	+	++	−	−
Vivipara...........	−	−	−	−	++	+
Busycon............	−	−	−	−	+	++

The tests were made by the interfacial (ring) technic. The symbol − means no reaction, ? means doubtful reaction, + moderate reaction, ++ very strong reaction.

Unfortunately it was impossible to estimate accurately the dilution of spider serum which resulted from bleeding the spiders used in this experiment. It was probably around 1 : 10 or 1 : 15. This would imply that the blood of the black widow spider contains 3 to 5 per cent of hemocyanin.

The author wishes to express his best thanks to Dr. F. E. D'Amour for the gift of one hundred black widow spiders for this work.

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THE DIURNAL MIGRATION OF DEEP-WATER ANIMALS

J. H. WELSH, F. A. CHACE, JR., AND R. F. NUNNEMACHER

(From the Woods Hole Oceanographic Institution[1] *and the Biological Laboratories, Harvard University)*

The diurnal vertical migration of planktonic organisms in the sea is a well-known phenomenon (Russell, 1927; Clarke, 1933; 1934).[2] Many more of the actively swimming animals, such as copepods, are found nearer the surface at night than during the day. For example, the level of maximum numbers of adult *Calanus,* in the Gulf of Maine, may be around 40 meters during the night while during the day it may be below 120 meters (Clarke, 1934). It is generally agreed that the most important external factor which regulates these daily movements is light. The greatest depth to which light may have an effect, however, is not known, since almost all studies have been confined to relatively shallow water. Obviously it would depend on the locality and the organisms being investigated, for an animal living at a considerable depth in a region where the water was very transparent might be influenced by the penetrating light, while another organism at the same depth in a region of low transparency would be unaffected. If diurnal migrations occur in deep water they must be considered in any study of vertical distribution. The lowest level at which they occur might be a satisfactory measure of the lower limit of the photic zone. These and other problems of the biology of the deeper water organisms have a direct relation to the possible vertical movements which may occur at levels deeper than any which have been adequately investigated. For these reasons a study was begun of diurnal migrations in deep water during a cruise of " Atlantis " to the Sargasso Sea in 1936.

During the cruise of the " Michael Sars " enough data were obtained from hauls with open nets to indicate that certain of the deep-water fishes and decapod crustaceans were to be found at higher levels during the night than during the day (Murray and Hjort, 1912). The " Michael Sars " data, however, did not indicate how deep such move-

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[1] Contribution No. 145.

[2] A rather complete list of references may be found in these papers.

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ments might occur, and are conclusive for only a few species of crustaceans and fishes.

The Sargasso Sea was selected for the present investigation since collections with closing nets were being made in this region, and because of the transparency of the water. Helland-Hansen (1931) had found that photographic plates exposed at a depth of 1,000 meters in waters between the Canary Islands and the Sargasso Sea were blackened after an exposure of 80 minutes. Clarke (1936) had calculated from data obtained by Clarke and Oster (1934) that there is sufficient light to enable deep sea fishes to see objects at a depth of 430 meters or deeper in the Sargasso Sea, depending on certain assumptions regarding their visual acuity and spectral sensitivity. Light of an intensity sufficient to affect the general activity of an animal would be found below the level at which objects could be seen.

TABLE I

Showing depths, time of day and duration of hauls made with closing nets at "Atlantis" Station 2667.

Haul	Depth	Date	Nets Open	Nets Closed
	meters			
1	400 800	Sept. 6	6:52 a.m.	7:52 a.m.
2	400 800	Sept. 7	4:07 a.m.	5:10 a.m.
3	400 800	Sept. 7	6:57 a.m.	7:57 a.m.
4	400 800	Sept. 7	9:31 a.m.	11:31 a.m.
5	400 800	Sept. 7	1:18 p.m.	3:18 p.m.
6	400 800	Sept. 7	5:10 p.m.	7:10 p.m.
7	400 800	Sept. 7	8:57 p.m.	10:57 p.m.
8	400	Sept. 8	12:39 a.m.	2.39 a.m.
	800	Sept. 8	Net attached improperly, failed to fish.	

Since it was desirable to make as many hauls as possible in a twenty-four-hour period only two closing nets were used and they were set to tow at 400- and 800-meter levels. The location of the station (2667) was 35° 40′ N. and 69° 36′ W. The duration of each haul and the time of day may be seen in Table I. One haul was made on September 6 with the nets open at 6:52 A.M. and closed at 7:52 A.M. A rough sea prevented further use of the nets that day but tows were resumed on September 7. The first three hauls were each of one hour duration, but the absence of salps and other forms which sometimes prevent longer tows, and the relatively small catches made it possible and desirable to increase the time of towing of subsequent hauls to two hours. In order to compare the catches the numbers of organisms taken in hauls 1, 2 and 3 have been doubled.

Since a modification of the closing nets developed and described by Leavitt (1935) were used for these hauls, and since they worked most successfully they will be briefly described and figured. The method of opening and closing was the chief feature of this net which differed from the one used by Leavitt. The releasing devices were the same. The changes were made by Mr. Nunnemacher with the advice of Dr. H. B. Bigelow.

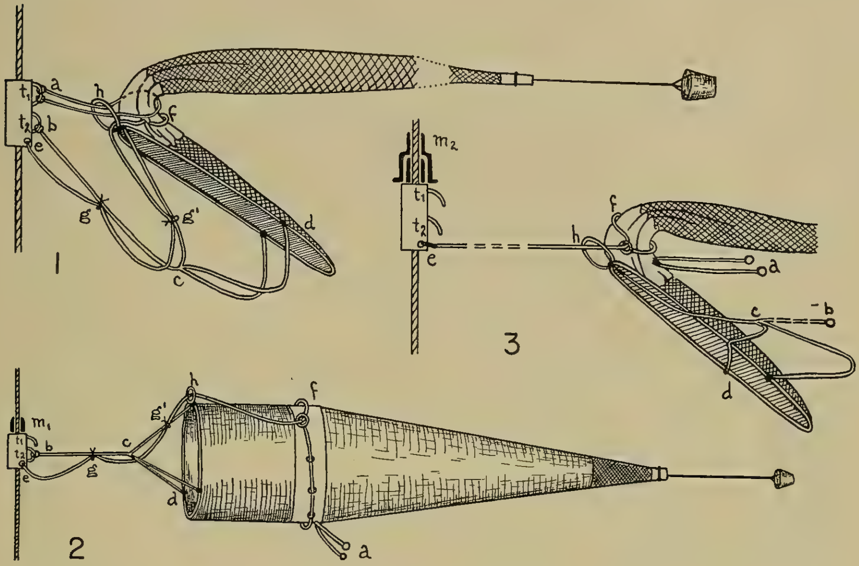

FIG. 1. Net closed, descending.
FIG. 2. Net open, towing.
FIG. 3. Net closed, ascending.

a. Fetter rope.
b. Single towing rope.
c. Three-point towing bridle.
d. Attachment of bridle on two-meter ring.
e. Attachment of pursing rope.
f. Pursing rope around belly-band of net.
g g'. Light string stops.
h. Small iron ring.
m_1. First messenger.
m_2. Second messenger.
t_1. Primary release.
t_2. Secondary release.

The accompanying figures show a net: (1) closed, descending; (2) open, towing; (3) closed, ascending. The net itself has a diameter of 2 meters at the mouth and is laced to a strong galvanized iron ring of 2 meters diameter. The sides of the net are made of stramin (6 threads per cm.) and are parallel for the first 2 meters and then sewed to a 60 centimeter wide, heavy canvas belly-band to which six 5-centimeter brass rings are fastened at equal distances over the lower half of the band. From the rear edge of the belly-band the net tapers from a 2-meter diameter to one of 25 centimeters over a distance of 7 meters. The end is finished off with another canvas band. Into the end of the net a silk net of corresponding taper is sewed so that it lines the last meter of the stramin and prevents excessive chafing of the catch.

On lowering the net (Fig. 1) a short fetter rope (*a*) fastened at its mid-point to the bottom of the belly-band tightly encircles the folded canvas, passes through the small iron ring (*h*) which is attached to the rim of the net and both ends of the fetter rope are hooked into the primary release. The releasing devices were those previously used by Leavitt (1935) and they proved entirely satisfactory. The loose towing and pursing ropes are " stopped " (loosely tied with cotton string) at points *g* and *g′* to make fouling impossible. After lowering the net to the desired depth, the primary release is opened by the first messenger and the fetter rope easily falls out of the way below the net. The net in opening merely unfolds, the loop of the pursing rope having been previously opened to the diameter of the net at the belly-band and then folded up with it. Figure 2 shows the net being towed by the strong three-point towing bridle and the pursing rope loosely surrounding the belly-band and " stopped " to the towing bridle. A bucket is also shown tied to the end of the net to prevent the tail from fouling in the eddies behind the closed net. After the required time of towing the second messenger releases the towing bridle which falls out of the way. The pursing rope, securely fastened to the cable, now takes the strain, constricts the net at the belly-band and effectively closes the net (Fig. 3).

At each haul an open net was attached 50 meters below the lower closing net. Since the level of maximum numbers of certain of the red prawns and blackfish is near 850 meters in this region of the Sargasso Sea, at least during the day, large catches were always taken by the open net and this material is being used in connection with other studies such as the eyes of deep sea crustaceans (Welsh and Chace, 1937). With the method we used to determine the depth at which a closing net fishes it is impossible to be accurate within about ± 100 meters at a depth of 800 meters. One can keep the level of the nets relatively constant by so controlling the speed of the ship as to maintain a constant wire angle. But it is not possible to know very accurately the level of fishing because of certain unknown forces which affect the catenary of the wire. We used the calculations made by Leavitt for setting the nets at 400- and 800-meter levels. These were based on the length of towing cable out, the pull of the nets when open, the weight at the end of the towing cable and the wire angle at the surface.

After sorting the hauls it was evident that some forms had not been taken in sufficient numbers to allow any definite conclusion to be drawn regarding their behavior. Others have not yet been identified. But several of the prawns of the families Acanthephyridæ and Sergestidæ, one of the chætognaths, and the copepods and fishes were present in considerable numbers and the data for these will be presented. The copepods were present in such very large numbers in some hauls at the 400-meter level that their numbers were estimated in those hauls and no attempt was made to separate and identify the several species. Fishes at 800 meters were also present in large numbers and counts were made of these in each haul but again they were not separated into genera or species. In spite of dealing with mixed populations of copepods and fishes, the data readily convince one that the more abundant species behave in a consistent manner and that their movements are obviously related to day and night. A remarkable correlation in the numbers of copepods and fishes in the separate hauls at the 400-meter level will be discussed later.

In the graphs the total number of animals of a group such as copepods, or of a species, where separations into species were made, taken in any haul is shown by a vertical bar. Haul 1 made on September 6 from 6:52 A.M. to 7:52 A.M. serves as a check on the consistency of catches from day to day as another haul was made on September 7 from 6:57 A.M. to 7:57 A.M. The lined bars represent the September 6 catches. When no representatives of a given species were present the word "none" appears in place of a bar. The net at the 800-meter level at the last haul made on September 8 from 12:39 A.M. to 2:39 A.M. failed to work as it was improperly attached; hence no data are available for this time and depth. The time of sunrise was 5:37 A.M., of sunset 6:19 P.M. at the position of this station on September 6. A heavy line at the bottom of each graph indicates the hours between sunset and sunrise, but it should be remembered that at any considerable depth the period of relative darkness would be longer than at the surface (Clarke, 1934). It should also be noted that haul number 6 began before sunset and continued some time afterward. Further remarks concerning a particular species or group of animals may best be made under separate headings.

Acanthephyridæ (Fig. 4)

The complete absence of any of the large red prawns from hauls made during the day at 400 meters was the first reassuring evidence that our results would add more convincing weight to the conclusions reached by Murray and Hjort (1912). *Systellaspis debilis* and *Acanthephyra purpurea* had been taken previously in this region of the Sargasso Sea (Welsh and Chace, 1937) but in these earlier collections with closing

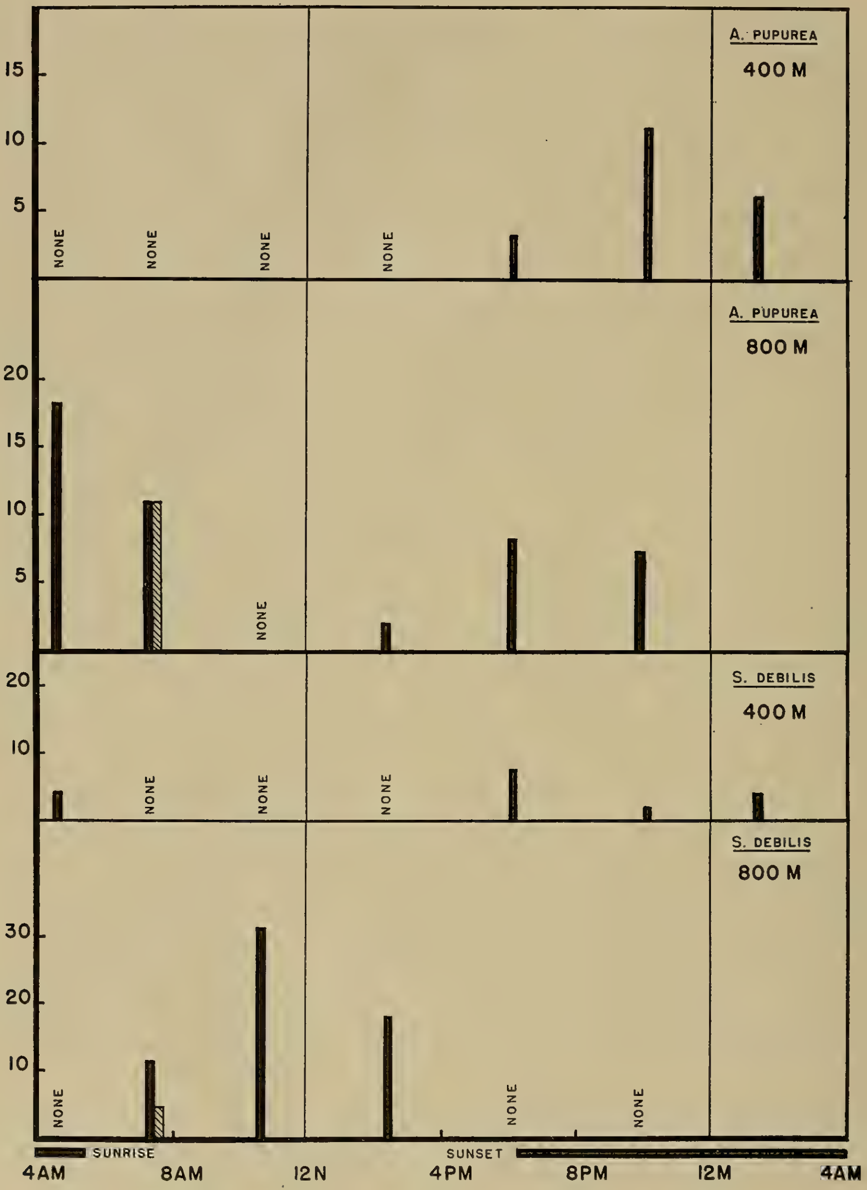

Fig. 4.

nets little attention had been paid to the time of day when the hauls were being made. At 800 meters the largest catch of *A. purpurea* was made in the early morning before sunrise, none were taken in the haul made nearest midday. On the other hand, *S. debilis* was taken at 800 meters only during daylight hours. Since it is known that *S. debilis* occurs at a somewhat higher level than *A. purpurea* a comparison of the catches of these two forms at the two levels strongly suggests that during the day the majority of *S. debilis* descend to a depth around 800 meters and the majority of *A. purpurea* to a somewhat greater depth.

Both *A. purpurea* and *S. debilis* have well-developed eyes. In contrast another acanthephyrid, *Hymenodora glacialis,* with degenerate eyes, has never been taken with closing nets from " Atlantis " at levels higher than 1,000 meters. It is an example of a form which has become adapted to a region below that at which penetrating sunlight has an effect on behavior and the development of the eyes. The upper limit of its distribution suggests that 1,000 meters is approximately the depth to which a biologically significant amount of sunlight can penetrate in water as transparent as that of the Sargasso Sea.

Sergestidæ (Fig. 5)

Of the sixteen species of *Sergestes* recorded from the North Atlantic, thirteen were taken at Station 2667. Of these thirteen, three species were taken in numbers large enough for statistical study. *Sergestes sargassi* appeared only in hauls at the 400-meter level and then only in those made during daylight hours. It was most numerous in the hauls made soon after sunrise. As this is known to be a comparatively shallow water species, it appears that it moves toward the surface during the night and returns to a depth around 400 meters during the day.

S. atlanticus was taken at the 400-meter level in two night hauls and in the haul which extended from about an hour before sunset until an hour after. None were taken at this level during the day. For some reason no sergestids of any species were taken in haul 7 at the 400-meter level in the late evening. At 800 meters *S. atlanticus* appeared in considerable numbers in the hauls made during the day, but except for one specimen which was taken in haul 2, none appeared in the other night hauls. Evidently this species migrates to deeper water during the day than does *S. sargassi.* It should be added that *S. atlanticus* has been taken in surface hauls made during the night.

The catches of *S. corniculum* at 400 meters were very much like those of *S. atlanticus* as regards relative numbers and times of appearance. At 800 meters, however, *S. corniculum* was found in relatively large numbers in all hauls during both day and night. It would appear

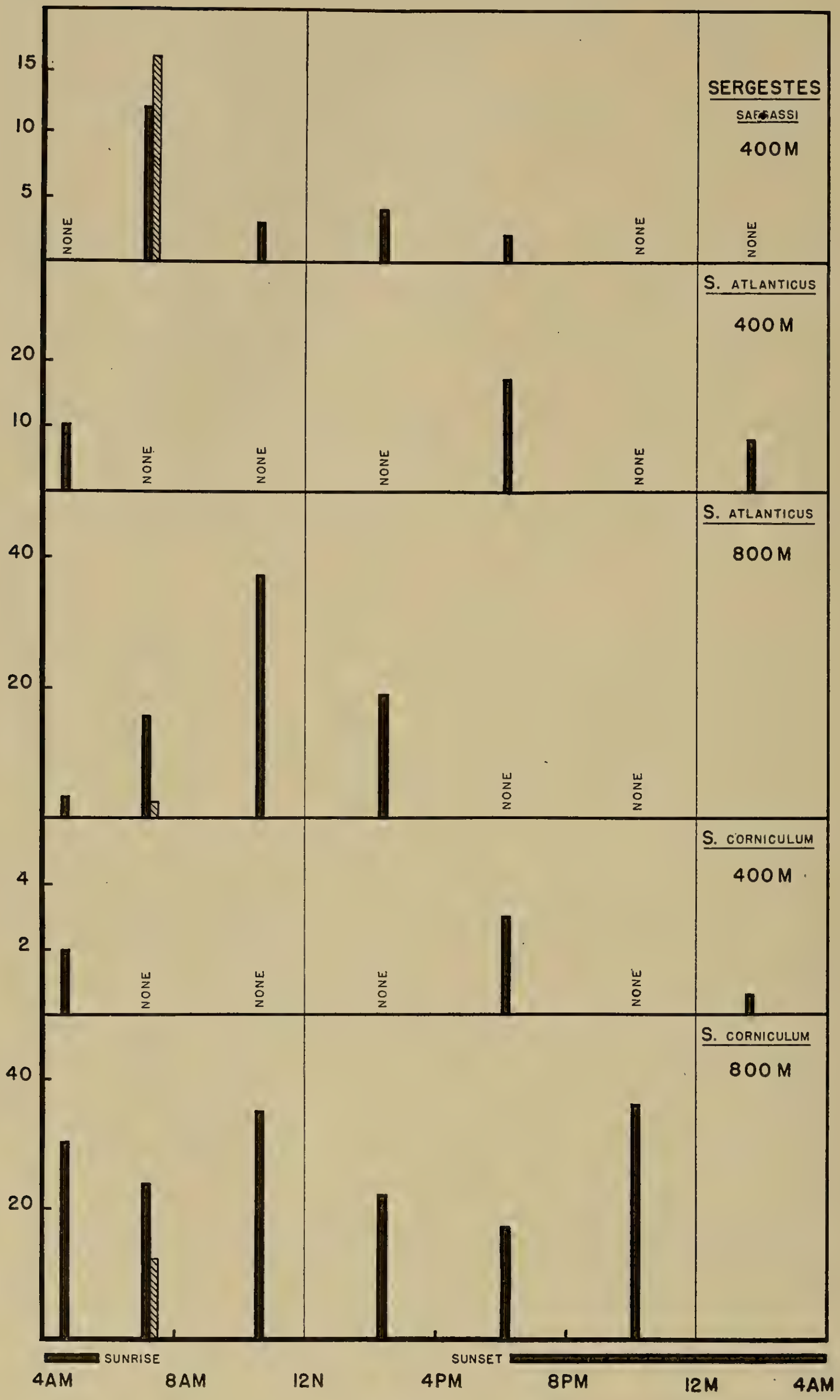
SERGESTES
SARGASSI
400 M
S. ATLANTICUS
400 M
S. ATLANTICUS
800 M
S. CORNICULUM
400 M
S. CORNICULUM
800 M
15
10
5
20
10
40
20
4
2
40
20
NONE
SUNRISE
SUNSET
4AM
8AM
12N
4PM
8PM
12M
4AM

FIG. 5.

that it was a form that migrated less extensively than some of the other species of *Sergestes*. That there are sergestids which are found below a level to which a sufficient amount of light penetrates to cause any diurnal vertical movements is known from our records on *S. mollis*. This species has been taken with closing nets only at depths of 1,600 meters and greater. Thus we see in this series of sergestids that the level of maximum numbers depends on the species and the time of day or night; also that the average depth at which a given species may be found depends on the extent of daily migration, if it occurs at all.

Copepods and Fishes (Fig. 6)

Because of the number and variety of fishes they have not been separated into species and neither have the copepods. These two distinctly different types of animals show, at the 400-meter level, a very similar type of distribution at different times of day and night, and this correlation may be seen if the graphs in Fig. 6 are examined. The largest catches were taken at the 400-meter level in the haul made just before sunrise and the one which extended from about an hour before until an hour after sunset. Most of the other catches when compared with these were small. It is probable that the large catches were taken as the migrating copepods and fishes passed the 400-meter level on their ascent and descent. The very large catches before sunrise must mean that these animals anticipate the return of dawn and start downward before there is any increase in light intensity. When the numbers of fishes taken at 400 and 800 meters are compared it is first obvious that many more were taken at the deeper level, and then that many more were taken during the day than during the night. It is probable that this depth is not very far from the daytime level of maximum numbers of deep-water fishes of this region. The striking correlation in numbers of copepods and fishes at the 400-meter level suggests that the fishes move with the copepods and thereby stay near their chief food supply.

Sagitta hexaptera (Fig. 7)

One large, easily recognized chætognath, *Sagitta hexaptera*,[3] was separated and counted and will serve as another example of a form which was taken only at 400 meters. On examination of the graph of the distribution of this form it is seen that the largest catches were made near midday, the smallest in the late afternoon and early evening. It would seem that the level of maximum numbers of this species was in

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[3] Identified by Miss Alice Beale.

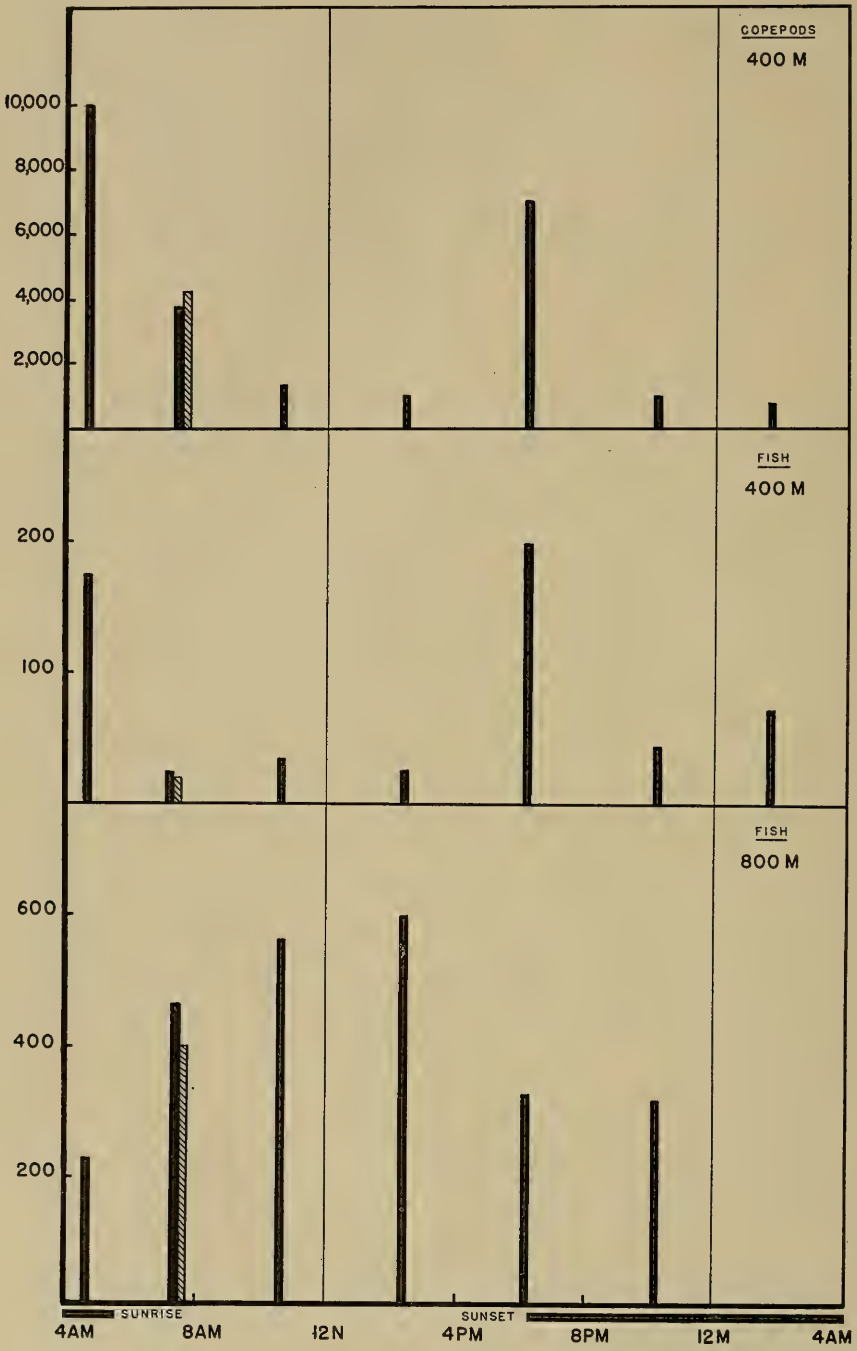
COPEPODS
400 M
10,000
8,000
6,000
4,000
2,000
FISH
400 M
200
100
FISH
800 M
600
400
200
SUNRISE
SUNSET
4AM
8AM
12N
4PM
8PM
12M
4AM

Fig. 6.

water shallower than 400 meters during the night and that they descended to near this level during the day.

Day to night differences in the numbers of certain species and groups of organisms, at depths of 400 and 800 meters, indicate not only that the animals of the deeper water of the Sargasso Sea migrate as do those of the shallow water, but that for certain species these daily migrations may be over a considerable distance. Light is probably the only day to night variable at a depth of 800 meters, or approximately one half mile; hence differences in light intensity must be largely responsible for these movements. There is, however, some indication that deep-water animals anticipate sunrise as Clarke (1934) found certain shallow water forms such as *Metridia* to do. This suggests that a physiological rhythm may in part be responsible for their behavior. Diurnal rhythms are known to persist for considerable lengths of time in the absence of regularly recurring changes in light intensity (Welsh, 1936). An internal cycle resulting in periodic changes in activity would account for the downward movement which begins before there is an increase in light intensity.

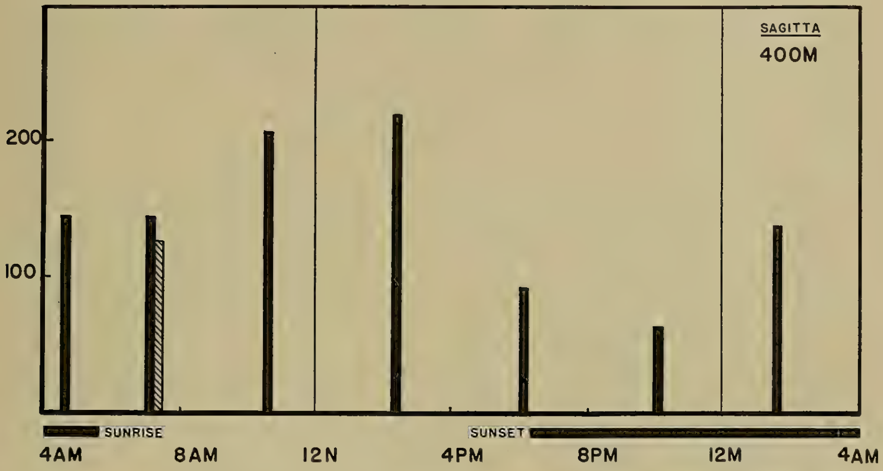

FIG. 7.

Many questions have arisen which can be answered only by further investigations. It will be of interest to determine the greatest depth at which daily migrations occur and to correlate these movements with measurements of light intensity. It will be necessary to make collections at many more levels before final conclusions can be drawn regarding the behavior of the organisms discussed in this paper, and of the many which have been omitted from this discussion. Possible differences in the behavior of animals with and without light organs should be investigated. The lower limit of the penetration of light of an intensity significant in determining the vertical distribution of animals should be correlated with the rather marked changes in temperature, salinity, oxygen and phosphates which occur at depths near 1,000 meters in certain parts of the Sargasso Sea. A combination of factors is doubtless responsible for keeping the majority of the macroplankton above 1,000 meters in this region, but it would be of interest to know which one, if any, is most important.

The collection and analysis of data of this kind is inevitably slow, but the vertical movement of organisms in the sea is of practical as well as of theoretical importance since the nightly aggregation of organisms near the surface increases greatly the potential food supply of surface fishes. Further work in the Sargasso Sea is planned and it should be possible to obtain a much more complete idea of the biology of deep-water organisms than we now possess.

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THE PROBLEM OF A PHYSIOLOGICAL GRADIENT IN MNEMIOPSIS DURING REGENERATION[1]

B. R. COONFIELD AND A. GOLDIN

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Experiments on certain ctenophores have shown the presence of physiological gradients in these animals. According to Child (1933) these animals show a decreasing susceptibility to toxic agents from the aboral to the oral end of a plate row. There are also definite indications that *Mnemiopsis leidyi* exhibits an aboral dominance in certain aspects of its activity (Coonfield, 1934 and 1936*a*). In addition to these reactions, it is known that this animal will regenerate readily (Coonfield, 1936*b*). All of these evidences suggested the advisability of making certain experiments on *Mnemiopsis leidyi* to test for a physiological gradient during regeneration and in response to grafts.

The experiments reported herein were of three types. In one, the time of regeneration following the removal of the aboral zone was recorded; in another, the time of regeneration in the plate rows was observed; and, in a third experiment, the reactions of *Mnemiopsis* to grafting of an apical organ was followed. Similar experiments have been used previously by Watanabe (1935) on *Euplanaria dorotocephala* and by Child (1935) on *Corymorpha*. The method of keeping *Mnemiopsis* during the experiments and of observing these animals was the same as reported by Coonfield (1936*b*). The temperature of the sea water in which the experimental animals were immersed ranged from 19.0° to 20.5° C. Each type of experiment is described and the results are given according to the headings: regenerating oral pieces, regenerating rows, and absorption of apical organ grafts.

REGENERATING ORAL PIECES

The purpose of these experiments was to record the time at which oral pieces of *Mnemiopsis* would regenerate organs which had been removed by a single cut across the body. These cuts were made at four levels: *A, B, C,* and *D* (Fig. 1). By these transverse cuts certain organs (Fig. 1) were removed along with the aboral portion of the body. We have assumed that regeneration was complete just as soon as the lost organs had been reformed. At this time the experimental animals had not regained their former size, but we believe that only growth is necessary for this to be accomplished. The results of these experiments are shown in Table I.

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[1] Contribution No. 23 from the Department of Biology, Brooklyn College.

REGENERATING ROWS

In the experiments reported here, the time required by rows to regenerate removed sections was recorded. A section including ten plates was removed from each of the four adtentacular rows at four different levels of the body. Level 1 begins at the apical organ and extends aborally; level 2 begins at the apical organ and extends orally; level 3 begins at the oral end of level 2 and extends orally; and level 4 begins at the oral end of level 3 and extends orally. Levels 1 and 2 were removed from the left and right rows respectively on one side, while levels 3 and 4 were removed from the left and right rows respectively on the other side of the animal. The healing and regenerating processes which followed the operations have been described adequately by Coonfield (1937*a*). The results of these experiments are shown in Table II.

TABLE I

Time in days required for aboral regeneration

Levels	Number operations	Number survived	Number regenerated	Average time
A	40	36	36	4.0
B	30	28	28	3.0
C	55	39	39	3.0
D	59	46	46	3.7

ABSORPTION OF APICAL ORGAN GRAFTS

In previous experiments (Coonfield, 1936*a*), it was found that apical organs which had been grafted to the surface of *Mnemiopsis* became absorbed by the host within a few days. The response of the host in these experiments suggested that this animal might be examined for a gradient by transplanting an apical organ to its body at different levels and observing the times at which the grafts are absorbed. Therefore a piece of an animal containing an apical organ, a portion of the infundibular funnel, and a part of the adradial canals was transplanted to the surface of another animal between its adtentacular rows. The levels are shown in Table III as 1, 2, and 3. Level 1 is just above the apical organ; level 2 is mid-way between the apical organ and the bases of the auricles; and level 3 is near the bases of the auricles. Usually these grafts established connections with certain canals of the host as described by Coonfield (1937*b*). In these the order of connections was with the adtentacular, the tentacular, the paragastric canals, and with the stomodeum (Fig. 2, *A*). In some, however, this order in connecting was not followed (Fig. 2, *B*). The results of these experiments are shown in Table III.

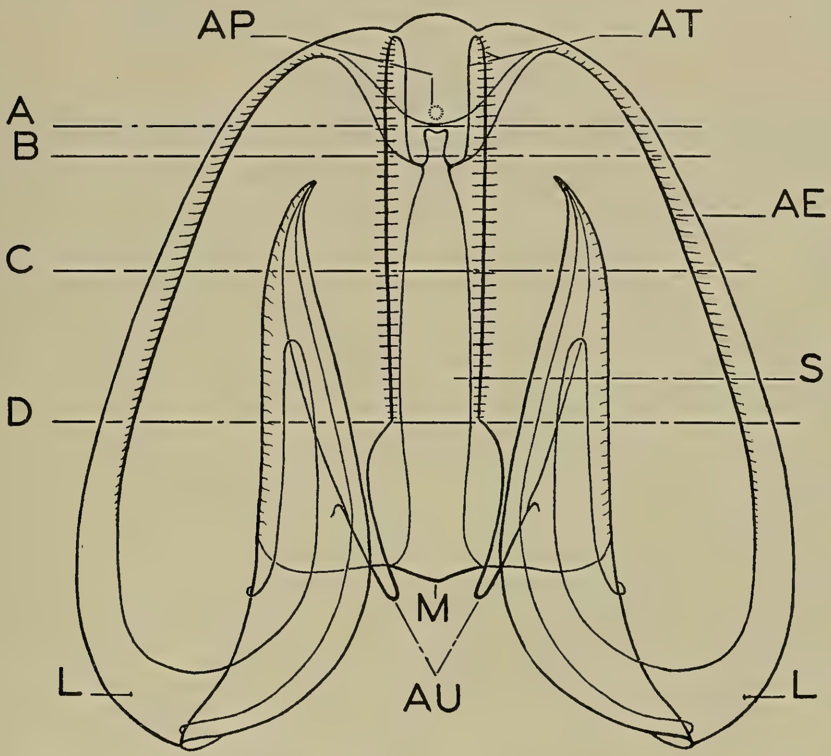

Fig. 1. This is a diagram of *Mnemiopsis* showing levels *A, B, C,* and *D* at which cross cuts were made. The organs as shown are: *AE,* adesophageal row; *AP,* apical organ; *AT,* adtentacular row; *AU,* auricles; *L,* lobes; *M,* mouth; *S,* stomodeum.

Since the apical organ is believed to exert a dominant influence over the other parts of the body of *Mnemiopsis* (Coonfield, 1936*a*), it is possible that this organ might be chiefly responsible for the absorption of the graft and would interfere with the detection of a physiological gradient. Therefore the aboral region including the apical organ was cut from specimens at the time that the transplantations were made.

TABLE II

Time in hours required for regeneration

Levels	Number operations	Number survived	Solid cord	Hollow canal	New plates
1	20	20	2.3	5.0	42.2
2	20	20	2.3	5.6	40.1
3	20	20	2.35	5.8	42.6
4	20	20	2.35	5.6	43.7

Controls were observed to detect any variation within groups of specimens. The results of the experiments are shown in Table IV. The levels indicated in this table correspond to the similar ones shown in Table III.

TABLE III

Time in days required for absorption of apical organ grafts. (The host was intact except for the transplantation.)

Levels	Number operations	Number survived	Number absorbed	Average time
1	17	8	8	9.25
2	17	14	14	12.25
3	16	8	8	10.50

TABLE IV

Time in days required for absorption of apical organ grafts. (Apical organ of the host was removed at time of transplantation.)

Level	Number operations	Regeneration		No regeneration		No absorption		No absorption No regeneration	
		Number absorbed	Average time	Number absorbed	Average time	Number regenerated	Average Time	Number	Time
2	24	7	11.6	5	11.4	2	10	10	7.1
3	15	2	12.0	2	9.0	3	6	8	8.1

DISCUSSION

Mnemiopsis will regenerate organs that have been removed and many of the changes taking place during regeneration can be observed easily (Coonfield, 1936*b*). Should a gradient be present during regeneration, there should be a difference in the time at which organs will regenerate. In making this test specimens were cut across at four levels and the regenerating oral pieces were observed. The progressive changes following these cuttings at these levels were very similar. This resemblance is illustrated in Figs. 3 to 10 where the arrows which point to a plate in a similar location on each of a series show that it is possible to believe that in Figs. 3 to 6 are represented four different stages of one animal. These figures represent four different animals at four different stages during regeneration. In Figs. 7 to 10 four other specimens which were cut at a lower level than those in Figs. 3 to 6 are shown. The results of these experiments (Table I) show clearly that there is no difference in the time of regeneration at the four levels of the body.

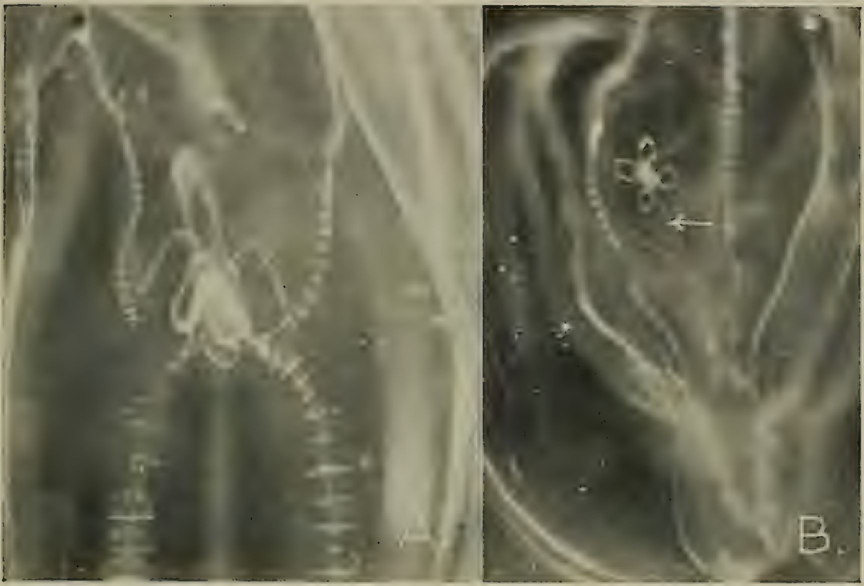

FIG. 2. These are photographs of the adtentacular surfaces of two specimens to which apical organs were grafted at level 3 (Table III). *A* shows the graft connected to two adtentacular canals of the host. *B* shows the graft connected to the tentacular canal (at arrow) of the host. Both photographs were taken five days after the operations.

Following the removal of sections from plate rows the remaining ends of the rows pulled together, fused, stretched, and formed solid cords which later became hollow as described by Coonfield (1937*a*). Later new plates formed in the healed region and in between the old plates which had been pulled apart by the stretching. The results of these experiments are shown in Table II. Here the average time in hours required for the formation of the solid cord of cells, the hollow canal, and the new plates is shown. There was no significant difference in the time of formation of these structures at the four levels of the body. This experiment is significant since the same organs of an individual were regenerating at the same time and at different levels. Furthermore there was no appreciable variation in the time of regeneration of these parts of rows when the results of experiments on individual animals were compared with each other.

Two methods of testing for a gradient by the absorption of apical organ grafts were employed. In one where the specimens were intact, only the time of absorption was involved. In the other where the apical organ of the host was removed at the time of the transplantation, the possible influence of this organ on the absorption of the graft was eliminated. The results obtained by the first method show that the grafts near the apical organ were absorbed at a slightly shorter time than those which were farther away from this organ. The difference in the time, however, is not enough to be significant. In those animals from which the apical organs had been removed at the time of the grafting (Table IV) the results show that there is no appreciable difference in the time of absorption of the grafts at different levels. It is clear also that a lower percentage of grafts was absorbed when the influence of the apical organ was removed. We believe that this low percentage of absorption was due to the absence of the influence of the apical organ. This influence is demonstrated by the delayed regeneration of an apical organ in those specimens on which the grafts persisted. We point out that in a relatively high percentage of these specimens the grafts persisted and there was no regeneration. We believe that the failure to regenerate was due to the inhibitory influence of the graft. In these specimens the grafts joined the various canals exactly as a normal apical organ is associated with the canals of the animal.

We conclude from the results of these experiments that a physiological gradient that has been shown to influence the times of regeneration

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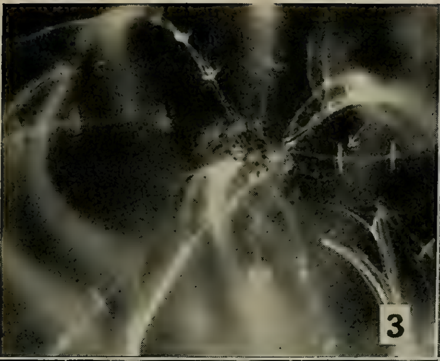
EXPLANATION OF PLATE I

FIGS. 3, 4, 5, and 6 are photographs of four specimens regenerating. They were all cut across at the same time and at level *A* (Fig. 1). They were photographed at 13, 39, 48, and 90 hours respectively after the apical ends had been removed. The arrows are pointing at similar plates on the four specimens to show the similarity of the regenerating processes.

FIGS. 7, 8, 9, and 10 are photographs of four specimens regenerating. They were cut across at the same time and at level *B* (Fig. 1). They were photographed at 13, 39, 48, and 90 hours respectively after the apical ends had been removed.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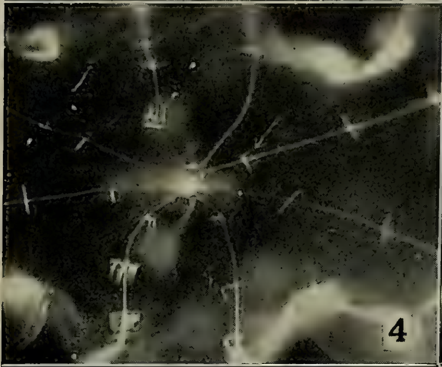
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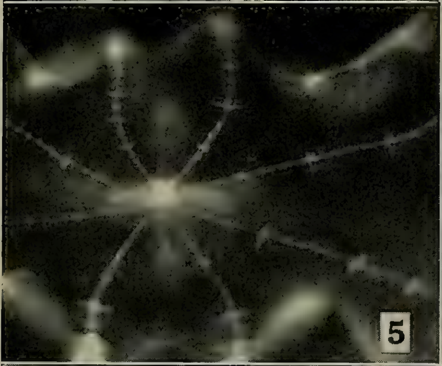
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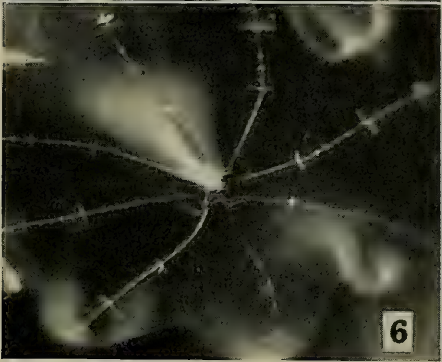
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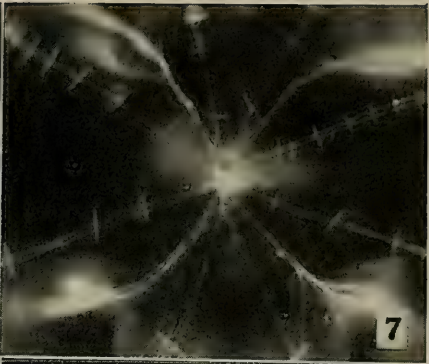
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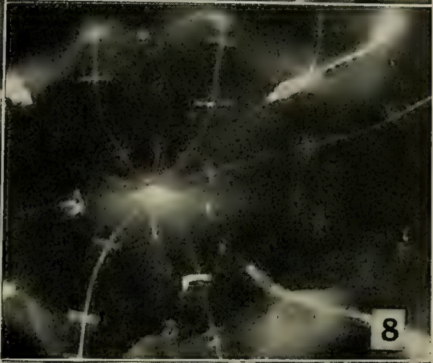
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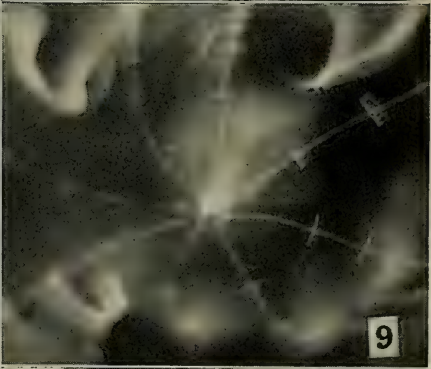
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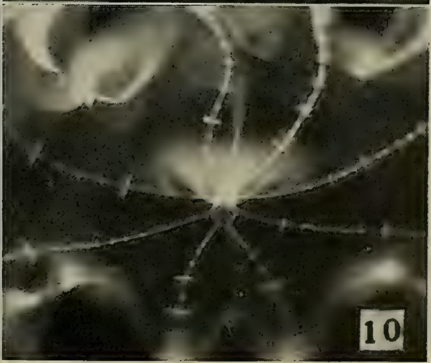
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PLATE I

and the absorption of organs in other animals is not present in *Mnemiopsis leidyi.*

Conclusions

1. A physiological gradient which would influence the times of regeneration and absorption of grafts is not present in *Mnemiopsis.*

2. The time of regeneration of oral pieces following transverse cuts at four levels of the body is the same at all of these levels.

3. The time of regeneration following the extirpation of sections of plate rows at four levels of the body is the same at all of these levels.

4. The time of absorption of apical organ grafts is the same at three different levels of the body.

5. Retained apical organ grafts either inhibit or delay the regeneration of an apical organ by the host.

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THE HEXOCTAHEDRON AND GROWTH[1]

OTTO GLASER AND GEORGE P. CHILD

(*From the Biological Laboratories of Amherst College*)

I

Kelvin's (1887) analysis of the conditions under which soap bubbles assume hexoctahedral form and together incorporate space without remnant, applies to tissue cells whenever these are surrounded and compressed on all sides by units essentially similar to themselves (Lewis, 1923, 1926, 1933). In all cases the more generalized the tissue and the more exact its quantification, the more closely the cell approximates a solid whose thirty-six equal edges bound eight hexagons and six non-contiguous squares.

II

Since cells and nuclei contain emulsions, we may anticipate, under appropriate conditions, either orthic or rhombic hexoctahedra among various kinds of protoplasmic inclusions. Indeed, Lewis (1926, p. 23) has verified Pouchet's (1847) discovery that polyhedra of this type occur among the yolk granules of a hardboiled egg. Here, despite much fortuity, we find practical realizations of the ideal form shown in Fig. 1. As Lewis has not depicted these polyhedra and Pouchet's figures are not easily available, a selection of coagulated yolk granules is reproduced in Fig. 2.

Comparable shapes, sometimes indistinguishable from orthic hexoctahedra, can be observed in dried cornstarch (Fig. 3). Many illustrations in Reichert's (1913) monograph indicate the same form, more or less modified, in other types of starch.

III

Translucent polyhedral bodies fill and often burst the nuclei in various tissues of "wilted" caterpillars (Fig. 4). In any given nucleus the polyhedra are remarkably uniform in size; in different nuclei they range from 0.5 to 15 μ in diameter (Glaser and Chapman, 1916).

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[1] We are indebted to Professor George W. Bain of Amherst College for photographs of the polyhedral bodies of the silkworm. Dr. Bain first drew our attention to the fact that these bodies do not polarize light. We wish to express our appreciation also to Dr. R. W. Glaser of the Rockefeller Institute for material sent in 1936 and for the privilege of studying the photographic plates made in connection with the original Glaser, and Glaser and Chapman investigations on the polyhedral bodies of insects.

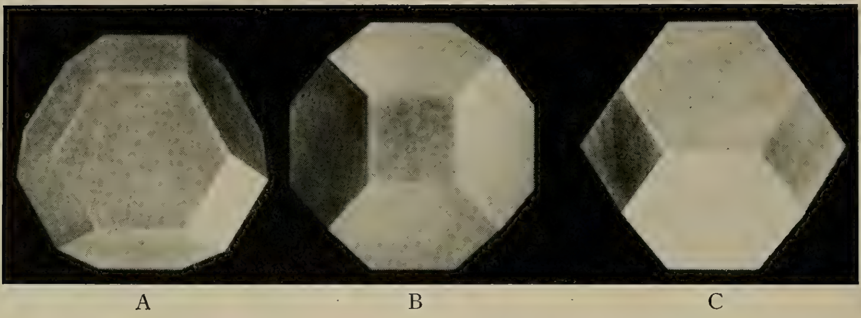

Fig. 1. Models of the hexoctahedron. In the originals, made of aluminum, the edges are exactly 2 cm. in length. *A,* with hexagonal facet toward the observer and lying on the hexagon, opposite. *B,* with square facet toward the observer and lying on the square opposite. *C,* interhexagonal edge or dihedral angle toward the observer and resting on the interhexagonal edge opposite.

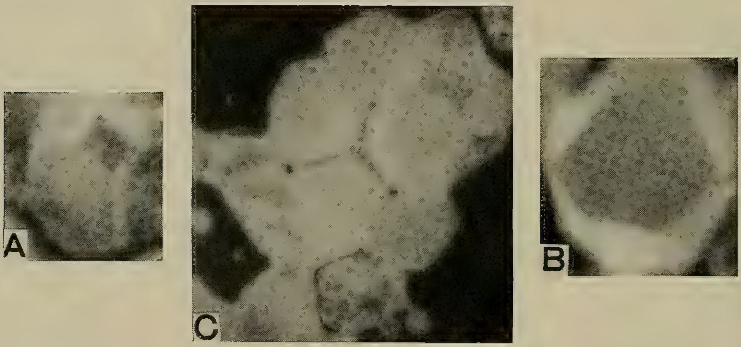

Fig. 2. Granules of coagulated egg-yolk under various illuminations and magnifications. No granule is completely in focus throughout.

A, B. Quadrilateral facets produced by truncation insufficient to realize equilateral edges.

C. A group of granules sufficiently uniform to illustrate practically ideal stacking. Focused on the planes of contact. The three dots are " waste " space.

Ocular recognition of form in three dimensions depends on optical sections combined with significant surface views. For the interpretation of these, oriented models (Fig. 1) are indispensable.[2]

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[2] Useful models can be constructed after the patterns of Matzke (1931). Matzke has also published a careful analysis of the orthic tetrakaidecahedron and its aggregation without waste of space (1927).

Experimentation shows that hexoctahedra, in air, are most stable when resting on their hexagonal faces. In this position, the surface nearest the observer is also hexagonal and between this proximal and the basal hexagon are two alternating tiers in each of which three lateral hexagons alternate with three squares (Fig. 1, *A*). In a second position, most frequent in viscous liquids, the polyhedron is supported by the overhanging edges of the lateral hexagons and comes to rest on a square face. Now the uppermost surface is also square and separated from its basal counterpart by two tiers of four hexagons each and an equatorial tier of four non-contiguous squares (Fig. 1, *B*).

To interpret microscopic polyhedra requires patience. Even though the facets are true planes, we cannot assume that every individual resting on a square or hexagon is also level. A specific polyhedron may deviate from the ideal, or have its basal facet tilted by neighbors or fragments of foreign material. Thus, an optical section may not conform with expectations based on simple square or hexagonal orientation.

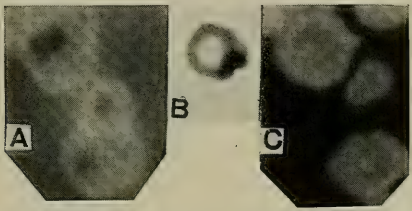

FIG. 3. Granules of dried cornstarch photographed by reflected light under various magnifications.

A. Upper left granule showing a clearly quadrilateral face; lower right, showing square facet, vaguely.

B. Lateral view of cornstarch granule showing two hexagonal facets; the lower one with a glitter point. To the right a quadrilateral face.

C. Similar to *B* but showing hila in hexagonal facets.

What then is required to establish the hexoctahedral form? Clearly, the object must be three-dimensional; must possess facets; and these, when not hexagonal, must be square. Squares cannot be contiguous. Facial angles, when not 120°, must be 90° and all edges must be equal. We should expect sections in which the hexagons are not equilateral and sections indicative of more than six sides. Octagons, decagons and dodecagons are all possible. Fragmentary outlines of the higher polygons are not easily intelligible.

Photographs, based on the two stable orientations of the models in Fig. 1 and including only samples of the typical surface views and optical sections most frequently encountered with the polyhedral bodies of the silk worm, are shown in Fig. 5. These records enable us to postulate the hexoctahedral form for the polyhedral bodies of "wilt."

If sufficiently familiar with the form, the observer can recognize the three-dimensional character of the polyhedra and indications of the two types of facet even at the lower magnifications shown in Fig. 4. To these views, we add the evidence secured by dark field and unilateral illumination. In favorable instances such lighting picks out some of

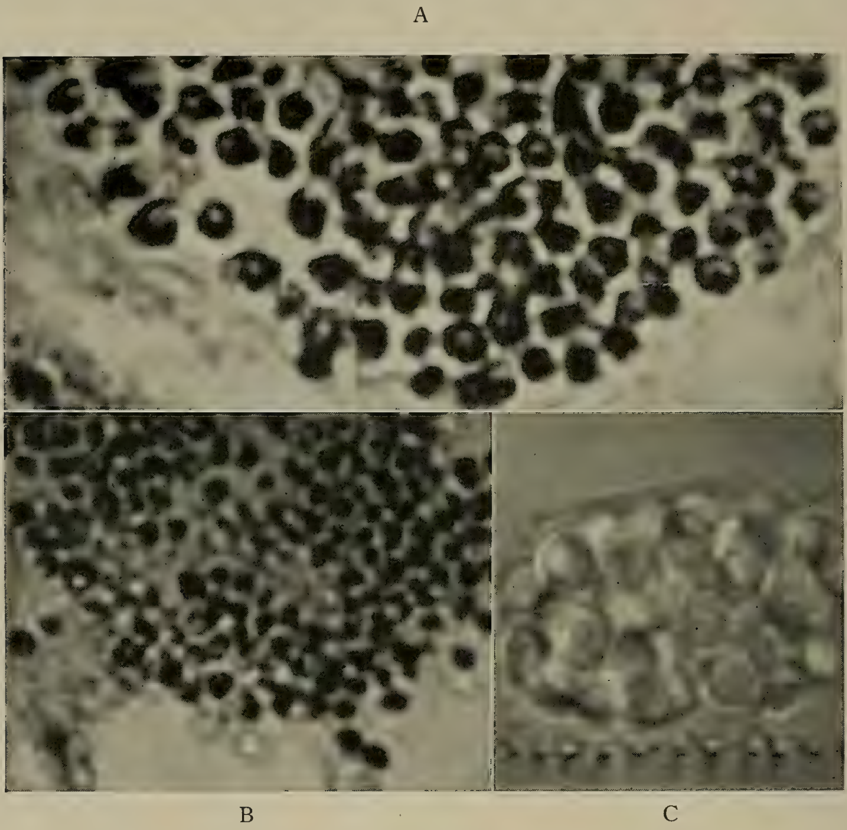

FIG. 4. Nuclei of wilted army worm and gipsy moth caterpillars.

Polyhedral bodies in three dimensions with numerous indications of facets in black or white and sharp dihedral edges. *A,* polyhedra in half the nucleus of a fat cell of an infected army worm × 1500: enlarged from the photographic plate reproduced in Glaser and Chapman (1916) as Fig. 3, Plate II. *B,* half of smaller nucleus of same × 1416. *C,* polyhedra in nucleus about tracheal tube of gipsy moth caterpillar × 1875: enlarged from the original reproduced as Fig. 2, Plate II, in Glaser (1915). As now printed, the above enlargements are reduced one-half.

the trihedral angles. These appear as minute points from which may radiate three slightly luminous edges. Photographs in which the objects are vaguely outlined by means of illuminated facets, trihedral angles, and edges are shown in Fig. 6. The hexagonal distribution of the trihedral angles and the square facets, each outlined by four luminous points, are especially noteworthy.

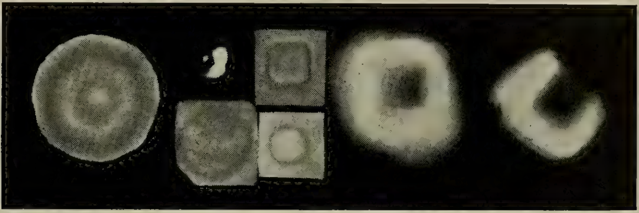

Fig. 5. Hexagonal and square orientations of polyhedral bodies.

The large hexagon is viewed by transmitted light. It is equilateral and the facial angles are 120°. The square orientations demonstrate equal sides with 90° angles. In many instances the upper levels of the adjacent lateral hexagons are partially lighted. The octagonal marginal outlines are visible whenever the optical sections cut hexagonal facets parallel to their diameters and square faces parallel to their diagonals.

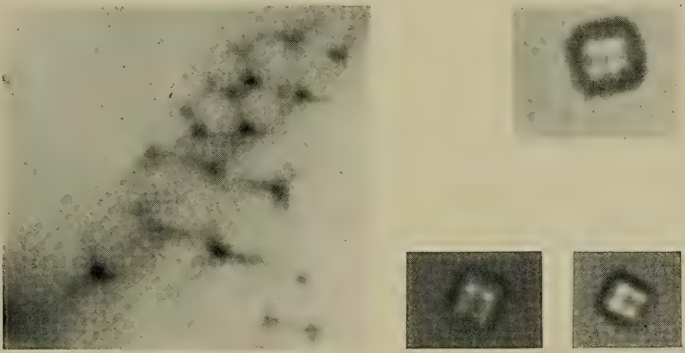

Fig. 6. Polyhedral bodies of the silk worm at various magnifications showing facets, trihedral and dihedral angles, under lateral illumination.

IV

For our immediate purposes it is unnecessary to know the chemical composition of the intra-nuclear hexoctahedra of insects, or whether these objects are crystals (*cf.* Glaser and Chapman, 1916). It is important that bodies with this shape stack without waste space; that slightly rhombic forms can be treated as if they were orthic (Kelvin, *op. cit.*); and that the properties of cellular emulsions and the pressures to which they may be subjected are such that hexoctahedra of all possible orders of magnitude and all degrees of perfection may be postulated. Many of these may be only rough temporary approximations; others may be expected whenever the emulsions of which they were parts are properly compressed and undergo fixation. Conceivably even true crystals may appear under conditions that would impose the same form on non-crystalline materials.

Systems of this sort may not be as regular throughout as the polyhedra within a given insect nucleus; nevertheless even yolk contains regions of essentially orthic units constant in size and presumably constant in weight. Whether aberrancies cancel out in sufficiently voluminous masses or whether the polyhedra or their precursors in a given system may deviate from the hexoctahedral ideal by constant fractions, the fact remains that irregularities prove relatively unimportant.

V

Stacked in n layers about a central unit, the total number of individuals, Sn, in an isogonic aggregate of orthic hexoctahedra is $4n^3 + 6n^2 + 4n + 1$ (Marvin, 1936). This statement is identical with

$$Sn + n - 0.5 = \frac{(2n+1)^3}{2}.$$

Essentially, therefore,

$$\log Sn = 3 \log (2n+1) - 0.3010.$$

With this transformation hexoctahedral aggregation assumes a formal identity with organic growth,

$$\log w = k \log (2t+1) + C,$$

in which weight is equated against time (Glaser, 1938, in press). Hence, if at some level within the cell, organic growth is controlled by hexoctahedral aggregation, layer number in such aggregates must be proportional to time.

VI

This proportion suggests an absolute standard for heterogonic correlations since the heterogony constant k_h in $y = bx^{k_h}$ is the ratio between the growth constants for x and y (Huxley, 1932; Glaser, 1938). Without identifying x or y with the units that control their magnitudes, it is possible to attribute the serial changes in x or y to a summation with properties indistinguishable from those of the hexoctahedral aggregation series. Accordingly, this series must be able to replace either organic correlative in the heterogonic equation.

When the ages of x or y are unknown, proper adjustment between the hexoctahedral series and the organic correlates may be possible by trial and error; when the organic weights are dated, the proportionality between layer number and time is decisive.

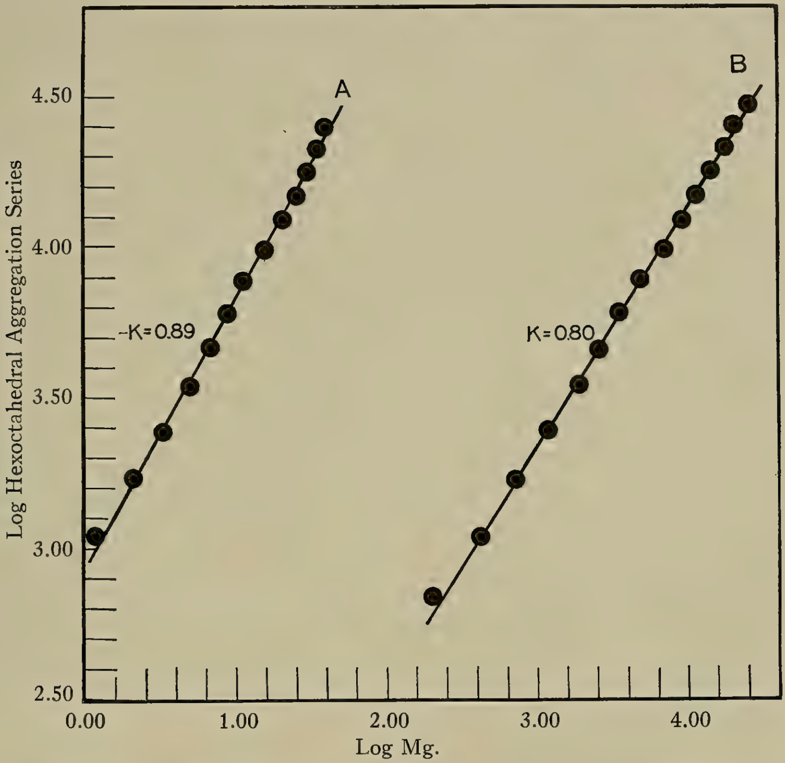

Fig. 7. Heterogonic plot. Two organic weight series against the ideal hexoctahedral aggregation series. *A*, the quantitatively organized chlorides in the chick embryo, based on Table I, Murray (1926, p. 794). *B*, fresh weight of chick embryos based on the original analyses of Murray (1926; Table III, p. 410).

Substitutions of the dated variety are illustrated in Fig. 7. Curve *A* shows the relation between the quantitatively organized chlorides of the chick embryo and the Marvin series; *B*, the relation between this series and the weight of the entire chick. In both instances the linear relationship implicit in $y = bx^{k_h}$ is fairly realized. Moreover, since the growth constant for the chick is 3.76 (Glaser, *op. cit.*, in press) the hexoctahedral heterogonic constant should be $\frac{3.00}{3.76} = 0.797$. The independent graphic determination yields 0.80. For the chlorides with a growth constant of 3.53 (Glaser, *op. cit.*) the constant for curve A should be $\frac{3.00}{3.53} = 0.849$. Independent graphic determination indicates 0.89.

Many other quantitative relationships of the types here illustrated can be predicted and, where the data permit, verified. For this reason we may consider the hexoctahedral aggregation series as an instrument which enables us to penetrate to analytical levels not attainable without reference to the geometrical foundations of organic cumulative growth.

SUMMARY

1. Certain starch grains and the polyhedral bodies of insects are hexoctahedra of the type described by Lewis for tissue cells and coagulated yolk, and by Kelvin in systems of soap bubbles.

2. Compression of units visible in cellular emulsions results in aggregates of hexoctahedra. Under the same conditions the same form is postulated as either imminent or actual, at levels below the limits of visibility.

3. If this extension applies to materials that control the dimensions of organisms, Marvin's hexoctahedral aggregation series should be capable of replacing either organic correlative in the heterogonic equation $y = bx^{k}{}_{h}$. Evidence is afforded by heterogonic curves in which the Marvin series is plotted against the chick embryo and the chlorides of the chick embryo.

4. The success of this test depends on two facts: (a) the heterogonic constant is the ratio between two growth constants; and (b) when isogonic aggregates of hexoctahedra are produced by organic growth, the number of layers in which the resulting units stack about a central unit, is proportional to time.

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STUDIES ON PRODUCTIVITY AND FERTILITY OF DROSOPHILA MUTANTS

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INTRODUCTION

From his tests of the mutant " Truncate " in various crosses, Hyde (*Jour. Exper. Zool.*, **17**: 173) concluded that the degree of fertility of each gamete was directly dependent upon the genes carried by that gamete, irrespective of the zygote producing it. On the other hand, the crosses carried out by Morgan (*Zeitschr. Abst. Vererb.*, **7**: 323) with the sex-linked recessive " rudimentary " had led to the hypothesis that " prematuration " effects of the zygote upon the developing germ cells explained the peculiar partial sterility of the rudimentary females. Accordingly, a study of the effects of gametic versus zygotic constitution on the productivity and fertility of various mutants was undertaken. These tests were started at Columbia University but were mainly made at Woods Hole during five summers there. I wish to express my sincere thanks to Professor Morgan and Dr. Bridges for their advice and help in this analysis.

PRODUCTIVITY OF VESTIGIAL

The first mutant investigated was the second chromosome recessive " vestigial wings " which had given evidence of low productivity not complicated by obvious sterility of any sort. In order to avoid high or aberrant mortality through overcrowding or underfeeding of the growing larvae, the individual females and their mates were transferred every few days to fresh culture bottles of large size liberally supplied with food. The total productivity of each female was obtained by continuing the transfer as long as the female lived. Table I gives summaries of the productivities of the wild stock control and of the crosses involving vestigial.

The most striking relation evident in the result is that the productivity is dependent primarily on the zygotic type of the mother. The cultures from vestigial mothers averaged 344 (51 vg ♀ with 17,538 offspring) not much more than half the average of 581 from non-vestigial mothers (72 non-vg ♀ with 41,897 offspring). But the average length of life of the vestigial mothers was 28 days while the average life of the non-vestigial mothers was 51 days. That the difference in productivity was mainly due to this difference in life-span was shown by the fact that the daily productivity averages 12.3 for the vestigial and 11.4 for the non-vestigial mothers.

In the results of Table I, a second striking relation is shown, viz.: that productivity rises with outcrossing to diverse strains. Thus, while the productivity of the females of the inbred Falmouth strains averaged 534, the productivity rose to 612 when outcrossed to another wild strain and to 611 when outcrossed to vestigial males. Similarly, the productivity of vestigial females of the inbred cross was only 283, but females of this same stock gave a productivity of 426 when outcrossed to Falmouth males and our intermediate productivity of 373 when outcrossed to F_1 heterozygotes. That the improvement with outcrossing is due to the genetic constitution of the hybrids as compared with the parental races seems clear and is analagous to the "heterosis" effect observed in corn strains. Further evidence of genetic influence upon productivity is seen in the fact that vestigials extracted from the F_2 gave an average productivity of 360—higher than the productivity of the vestigial stock. The improvement seen in the extracted vestigials must be due to genes that assort separately from the vestigial gene.

TABLE I

Crosses testing productivity of vestigial and wild

♀ ♂	Pairs	+ ♀	+ ♂	Vg ♀	Vg ♂	Total	Average Production
Fal. × Fal.	37	10,053	9.677	—	—	19,730	534
Fal. × W. H.	9	2,828	2,663	—	—	5,491	612
Fal. × vg	10	3,120	2,985	—	—	6,105	611
+/vg × vg	16	2,742	2,611	2,678	2,540	10,571	661
vg × vg	21	—	—	3,004	2,937	5,941	283
vg × Fal.	10	2,153	2,106	—	—	4,259	426
vg × +/vg	11	1,022	1,033	1,057	988	4,100	373
F_2vg × F_2vg	9	—	—	1,645	1,593	3,238	360

THE FERTILITY OF EGGS FROM THE VESTIGIAL CROSSES

Besides length of life and average daily output of eggs, another factor in productivity is the percentage of eggs that give adults the fertility of the eggs. Hyde had introduced the method of isolating counted eggs and observing how many adults these produced. Similarly, I studied fertility by isolating each female and her mates in a bottle and collecting her eggs upon a strip of blotting paper thickly spread with fermented banana. These strips were changed twice daily, the eggs counted and transferred to well-fed culture bottles where larvæ developed. By additional food supplied as the larvæ grew, mortality, in the post-egg stage, was minimized and the differences in egg fertility brought out clearly. In Table II are summarized the results of the fertility studies on crosses involving vestigial and the wild stock Falmouth.

TABLE II

Crosses testing fertility of vestigial and wild

♀ × ♂	Eggs	Adults	Fertility
			per cent
Fal. × Fal.	3,505	2,080	59
Fal. × +/vg	1,995	1,456	73
Fal. × vg	2,619	1,868	71
+/vg × Fal.	1,801	1,475	82
+/vg × +/vg	3,240	2,070	64
+/vg × vg	2,066	1,441	70
vg × Fal.	1,617	1,069	66
vg × +/vg	1,973	1,164	59
vg × vg	4,149	1,897	46
F_2vg × F_2vg	640	465	55

The significant feature of the results of Table II is the parallelism shown between the fertilities and the productivities of Table I. Thus the fertility of the Falmouth inbred stock was 59 per cent which was raised to 71 per cent and 73 per cent when these same females were outcrossed to vestigials or to heterozygotes. Similarly, the fertility of the vestigial females was raised from 41 per cent to 66 per cent and 59 per cent when outcrossed to Falmouth and to heterozygous males—the increase in fertility being similar in amount to the increases in productivity and presumably accounting for their occurrence. The fertility of the heterozygous females mated to wild males was markedly better (82) than that of the wild females (59) or vestigial females (66) mated to wild males. Finally, the fertilities of the F_2 extracted vestigials (55) was better than that of the inbred stock (46) and was matched by the increased productivity noted in Table I.

Hence, it may be concluded that the primary genetic difference between the vestigial and wild stock (the vestigial gene) was responsible for the curtailment of productivity of vestigial females to approximately half that of wild through its determination of a phenotype not able to function for much more than half the normal span of life. The two original stocks differed in secondary gene pairs which (1) heightened the productivity of crosses by improved viability of the hybrids in the egg stages, (2) heightened the productivity of hybrids (heterosis effect) and which (3) were able to redistribute with respect to the vestigial pair and bring about the improved fertility and consequent improved productivity of the F_2 extracted vestigials.

FERTILITY OF THE HIGH-PRODUCING "HAIRY" STRAIN

Unusually high production had been noted for a few strains, notably for the heterozygotes of hairy. A study of the fertility of hairy (Table III) showed that the outcross of hairy by Falmouth gave an increased fertility of 64 per cent as compared with the 59 per cent of Fal. × Fal. and the 60 per cent of hairy × hairy. The hybrids themselves gave an even higher fertility of 73 per cent corresponding to the unusually high productivity originally observed.

TABLE III

Fertility of hairy and wild

♀ × ♂	Eggs	Adults	Fertility
			per cent
Fal. × Fal.	3,505	2,080	59
h × Fal.	1,157	743	64
h × h	1,706	1,032	60
+/h × +/h	679	494	73
F_2 × h	4,527	2,747	61
F_2h × h	2,625	1,459	56

THE FERTILITY RELATIONS OF SINGED AND SINGED^3

Among the mutant strains at Columbia there were three which showed complete or nearly complete sterility of the females but for which there existed allelomorphic strains similar in phenotypes but with approximately normal fertility of the females.

One such set of allelomorphs was singed and singed^3, sex-linked recessive found by Mohr. In both mutants, the bristles and hairs are tightly curled or crumpled, but singed^3 has normal fertility while singed females lay few eggs all abnormal in shape and filaments.

As shown in Table IV, the abnormal eggs laid by young singed females averaged only about 7 per day as compared with 20 per day for singed^3 females and 25 per day for their hybrids. None of the eggs of the singed females gave offspring while the fertility of the eggs of singed^3 females was 33 per cent with brothers and 53 per cent in crosses to singed males. Fertility of the hybrids was 38 per cent and 37 per cent when mated to singed and singed^3 males—better than that of either pure race.

TABLE IV

Fertility of singed and singed ^{3}

♀ × ♂	Eggs	Eggs Daily	Adults	Fertility
				per cent
sn × sn..............	754	6.6	0	0
sn × sn^3..............	1,023	7.8	0	0
sn^3 × sn^3.............	3,272	20.4	1,085	33
sn^3 × sn..............	3,269	19.2	1,718	53
sn/sn^3 × sn...........	2,903	24.4	1,093	38
sn/sn^3 × sn^3..........	3,034	25.9	1,112	37

FERTILITY RELATIONS OF REDUCED AND SCRAGGLY

The second—chromosome-recessive allelomorphs "reduced" (rd) and "scraggly" (rd^s) both found by Bridges, are characterized by irregular and variable reduction in number and size of the bristles. Scraggly is of normal fertility but females of reduced lay only a few eggs which almost never give offspring though they are normal in appearance.

TABLE V

Fertility of reduced and scraggly

♀ × ♂	Eggs	Adults	Fertility
			per cent
rd × rd....................	460	0	0
rd × rd^s....................	540	4	1
+/rd × rd................	385	89	23
+/rd × rd^s...............	1,305	563	43
rd^s × rd^s..................	1,299	526	40
rd^s × rd...................	688	315	46
rd/rd^s × rd...............	3,983	1,162	29
rd/rd^s × rd^s...............	4,560	996	22

As shown in Table V, the eggs from reduced ♀ × reduced ♂ gave no offspring, while reduced ♀ out-crossed to scraggly ♂ gave only 4 offspring. That the poor fertility of reduced is semi-dominant seems probable from the sub-normal fertility of heterozygous rd/+ ♀♀ × rd ♂♂ (23 per cent) and × rd^s ♂♂ (43 per cent), though, as usual, the outcross gave the higher fertility. Similarly, the semi-dominance of the poor fertility of reduced may account for the fact that the rd/rd^s females gave only 29 per cent and 22 per cent fertilities, lower than the fertility of scraggly females (40 per cent inbred and 46 per cent outcrossed). Within each fertility test involving reduced or scraggly, a high degree of variability appeared, with a tendency for the fertilities of individual pairs to fall into three groups—one below 5 per cent, one centering around 20 per cent and one around 50 per cent.

FERTILITY OF RUDIMENTARY^7 AND RUDIMENTARY^{14}

The original mutation rudimentary had been lost, but several new allelomorphs had been found by Bridges. Among them, rudimentary^{14} had the very short wings and female sterility characteristics of the rudimentary, while rudimentary^7 had wings only slightly shorter than normal and fertility not obviously reduced. The eggs of both strains are normal in appearance.

TABLE VI

Fertility of Rudimentary^7 and Rudimentary^{14}

♀ × ♂	Eggs	Eggs Daily	Adults	Fertility
				per cent
r^{14} × r^{14}	2,390	13	0	0
r^{14} × r^7	2,485	14	3	0+
r^7 × r^7	3,016	18	1,449	48
r^7 × r^{14}	2,194	18	1,263	58
r^7/r^{14} × r^{14}	6,562	40	2,883	44
r^7/r^{14} × r^7	3,413	35	1,886	55

As seen in Table VI, rudimentary^{14} ♀♀ laid an average of 13.5 eggs per day, though none gave offspring in the mating to rudimentary^{14} ♂♂ and almost none in the outcrosses to rudimentary^7 ♂♂. Rudimentary^7 ♀♀ gave more eggs per day (18) and these had the nearly normal fertility of 48 per cent upon inbreeding and 58 per cent upon outcrossing to rudimentary^{14}. The r^7/r^{14} hybrids gave egg outputs of 39 ± per day, higher than either parent. The fertility of the hybrids, 44 per cent, and 55 per cent, show only a little lowering from the rudimentary^7 standard through the substitution of the infertile rudimentary^{14} gene whose effects are nearly recessive.

Correlated Variability of Phenotype and Fertility

The phenotype variability of vestigial, hairy, singed and singed^3 was very slight under the stable culture conditions of the experiments and correspondingly the productivity and fertility were highly uniform for the different individual mating within each type of cross.

The reduced and scraggly phenotypes showed considerable variability both in the bristly size and number and in the fertilities. Even more variable in phenotypic expression are rudimentary^7 and rudimentary^{14}, and the greatest variability in wing size and venation reduction was shown in the r^7/r^{14} hybrids. Corresponding fairly close to the degree of phenotypic departure was the reduction in productivity of the rudimentary^7 and r^7/r^{14} hybrids. Nine hybrids, selected for their extreme shortness of wings, gave 626 eggs from which only one offspring emerged—thereby resembling their rudimentary^{14} parent in both phenotypes and productivity.

Discussion

The results of the experiments are all compatible with the view that fertility of gametes is independent of their genic content but is determined by the phenotype of the zygote producing them. Even in normal races, a surprisingly high proportion of eggs, about 40 per cent, may fail to produce adults. It would seem that the stages of fertilization and early embryonic development must be highly unstable and precarious. That the genetic composition of the zygote influences strongly the early process of development is evident from the practically universal improvement in fertility and productivity brought about by outcrossing. Other evidences of the rôle of inheritance in the fertility—increased fertility of the hybrids and changed fertility of extracted types—point to a situation for *Drosophila* analagous to that in maize, where inbred strains differ by large numbers of genes with slight individual but larger cumulative effects.

THE pH OF SEA WATER AS MEASURED WITH THE GLASS ELECTRODE

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Most methods available for the determination of pH must be subjected to critical study when applied to sea water. The reason lies in the fact that sea water is a relatively weakly buffered medium with a high salt content. For instance, the application of the colorimetric method to sea water has required the evaluation of rather large correction factors for the so-called salt error. In the case of the quinhydrone electrode, the determinations of sea water are not only subject to a salt error but accuracy is also impaired by the inherent instability of quinone at the pH values encountered. The method of choice would appear to be the glass electrode since it is highly suitable to unbuffered solutions. It is, however, well known that the glass electrode is susceptible to salt effects particularly in the alkaline range. It is the purpose of this paper to show that the salt content of sea water does not interfere with the application of the glass electrode to the determination of its pH and that such determinations may be made with a fair degree of accuracy.

PROCEDURE

The method of reference in all pH determinations is the hydrogen electrode. It would therefore appear that the simplest procedure for checking the accuracy of the glass electrode would be parallel determinations on sea water by both procedures. Such a study is unfortunately open to experimental difficulty by reason of the fact that the CO_2 tension of sea water and thereby its pH is altered while developing the atmosphere of hydrogen necessary to the use of the hydrogen electrode. Though this experimental difficulty is surmountable by the use of special apparatus of the type described by McClendon (1917), it was felt that a simpler though valid test of the effect of sea salt on the glass electrode might be furnished by parallel determinations by both methods on sea water deaerated with hydrogen. Even though the pH of sea water so treated is altered toward more alkaline values a sample uniformly treated should serve for an adequate comparison of the two methods. It was, however, impossible to obtain with the hydrogen electrode consistent values upon sea water so treated. The poor buffering capacity of such samples may be responsible. An indirect attack upon the problem not unlike that used by Sørensen and Palitzsch (1910) in their standardization of the colorimetric method for use with sea water was therefore adopted. This involved the addition of an amount of dry sea salt to various buffer solutions so as to give a concentration of sea salt equal to that found in sea water. The pH values of the buffer solutions were determined both before and after the addition of the sea salt using both the hydrogen and the glass electrode. The sea salt was obtained by evaporation of sea water on a water bath, any organic matter that precipitated out during the heating was filtered off. The salt so obtained was dried at 130° C. A liter of sea water yielded 33.1 grams of dry salt.

The hydrogen electrode employed in the determinations was the Clark type shaking vessel. A saturated KCl-calomel half-cell was used which was standardized against a standard acetate buffer, which in turn had been checked against a 0.1 molal KCl-calomel half-cell. The tentative standard of potential proposed by Clark (1928) was used. The hydrogen used was deoxygenated by passing it over heated (850° C.) platinized asbestos. The potentiometer circuit was the orthodox one, a galvanometer being used as a null point instrument.

Two types of glass electrodes were used. What will be designated hereafter as Type I was a commercial apparatus known as the Beckman pH meter. In this glass electrode the glass membrane separates a quinhydrone electrode from the sample to be measured which in turn makes a liquid junction with a saturated KCl-calomel half-cell. The amplifier and potentiometer circuit were not available from the manufacturer. The outfit was used without any modifications. In the other apparatus (Type II) we employed a Leeds and Northrop, No. 7673 vacuum tube amplifier in conjunction with the potentiometer and galvanometer of the hydrogen electrode circuit. The glass electrode, which was blown from Corning 015 glass, separated a Ag-AgCl half-cell from the sample and a saturated KCl calomel half-cell. The junction between the sample and saturated KCl was made at a stopcock of the type employed by Stadie, O'Brien, and Laug (1931).

All determinations were carried out in a room which was automatically kept at a relative humidity of 50–60 per cent. Rigid temperature control was not maintained though the variation during a series of readings was never more than 0.1° C. The control of humidity was found to be an important item in the reliable use of glass electrodes of the type employed under the climatic conditions of the seashore.

RESULTS

In Table I are summarized the results obtained on buffer solutions with and without the addition of sea salt. Each pH value given for the hydrogen electrode is the average of triplicate determinations while the glass electrode results are the average of duplicates. The hydrogen and glass electrodes agreed within the experimental error in all cases except that of the borate buffer with added sea salt. Here the glass electrode readings tended to fall a little below those of the hydrogen electrode. This may be the result of a salt effect but it should be noted that the pH has shifted toward more alkaline values where the glass electrode usually begins to deviate below the theoretical. The pH shift produced by the addition of sea salt to the borate and phosphate buffers is partly to be attributed to the precipitation of some of the buffer components by the Ca and Mg salts present in the sea salt. This precipitate was filtered off before making the measurements recorded here. Another contributing factor to this pH change is undoubtedly the change in activity coefficients brought about by the higher ionic strengths of the solutions. This factor and the volume changes that may have occurred on addition of the dry salt to the buffer solutions, are largely responsible for the pH shift in the case of the acetate and HCl buffers since no precipitates occurred in these solutions.

TABLE I

The pH of buffer solutions as affected by the addition of sea salt.
Temperature, 25° C.

Buffer	Hydrogen Electrode	Glass Electrode No. 1	Glass Electrode No. 2
HCl	1.04	1.02	1.04
HCl + salt*	1.12	1.11	1.12
Acetate	4.60	4.59	4.61
Acetate + salt*	4.58	4.59	4.61
Phosphate	6.99	6.99	6.99
Phosphate + salt*	6.72	6.70	6.70
Borate	9.13	9.13	9.13
Borate + salt*	9.51	9.46	9.47

* Dried sea salt added to a known volume of buffer in amounts equal to that present in the same volume of sea water.

We feel that these results justify the direct use of the glass electrode for the measurement of the pH of sea water. In drawing this conclusion we are mindful of the fact that the precipitation of some Ca and Mg salts in the two most alkaline buffers has somewhat distorted the ion balance from that found in sea water. It is doubtful, however, that the presence or absence of such small amounts of these large divalent ions would modify perceptibly the behavior of the glass electrode when much larger quantities of the smaller univalent sodium ion have no pronounced effect. Dole (1937) has stated that " the cation error is greatest with those cations that most easily penetrate the glass, sodium and lithium being the worst offenders, while large ions, such as potassium, and also divalent ions have only a small influence."

TABLE II

The pH of sea water as measured with the glass electrode. All samples collected September 10, 1936, Woods Hole, Mass.

Sample No.	Source	Water Temperature	pH
		° *C.*	
1	Great Harbor * Laboratory Dock	20.0	8.13 8.14
2	Tank supply (Room 333)	20.0	8.05 8.05
3	Tank supply (Room 110)	20.0	8.05 8.05
4	Great Harbor * Laboratory Dock	20.0	8.14 8.14
5	Eel pond *	21.5	7.99 7.98

* Surface water.

In Table II are summarized a few determinations on different samples of sea water. These results were obtained with the Type I glass electrode. Of interest is the difference in pH between samples drawn from the tank system supplying the laboratories, the water for which is pumped from the harbor, and samples collected directly from the harbor. This difference is well outside the maximal experimental error (± 0.02). Since all samples were collected in the order given, this difference cannot be attributed to a sudden change in electrode behavior because Samples 1 and 4 are in good agreement. Moreover, the agreement obtained on

Samples 2 and 3 collected in different parts of the laboratory building would indicate that local contamination of the system was not responsible. Since the feed pipes are mainly of lead it is possible that the acid swing is due to the removal of carbonate as an insoluble lead salt. This seems unlikely, however, in view of the fact that such lead feed pipes after continued use accumulate a protective coating of such a precipitate which should prevent further interaction. Another possible explanation for the difference may be the fact that though the two sets of samples are taken from the same locality, one is pumped from a depth and the other is surface water. Though the two samples registered the same temperature at the time of collection, the water from the greater depth may have possessed a lower temperature before its passage through the storage and feeder system. If so, a more acid pH would be encountered due to the lag in the readjustment of the carbon dioxide equilibrium as the temperature increased, a process which Irving (1925) has shown to be slow. The greater acidity of the water collected from the eel pond is to be expected in view of the more stagnant condition of this body of water.

The glass electrode has been used by Mitchell and Taylor (1935) to measure the pH of sea water at different dilutions in order to determine the dissociation constant of cresol red in sea water of different salinity. This work was confirmed and extended and, in addition, data upon phenol red were obtained by Mitchell, Buch, and Rakestraw. Since these authors used a glass electrode with a thin film membrane in contrast to the heavier type employed by us, we wish to emphasize the fact that our proof of the validity of the glass electrode values on sea water applies to this type of membrane only. It would be desirable to have a similar study made with the thin film type of membrane.

SUMMARY

The hydrogen electrode and the glass electrode registered approximately the same pH values for various buffer solutions in which dry sea salt was dissolved to give a concentration similar to that found in sea water. A direct comparison of the two methods on sea water was not possible because the behavior of the hydrogen electrode was erratic in this poorly buffered medium. It is concluded that the salt content of sea water does not interfere with the application of the glass electrode to the determination of the pH of sea water and that such determinations may be made with a fair degree of accuracy (± 0.03 pH). The pH value of several samples of sea water have been determined and their differences discussed.

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THE RELATION OF ENDOCRINE FEEDING TO REGENERATION, GROWTH AND EGG CAPSULE PRODUCTION IN PLANARIA MACULATA

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An attempt was made to determine whether the time required for head regeneration in *Planaria maculata* following a series of decapitations would be altered by feeding beef endocrines. In view of the results of Wulzen (1923) on *P. maculata* and Castle (1928) on *P. velata* a record of the size of the individuals was kept. A preliminary report (Goldsmith, 1935) summarized the results; a more complete account follows.

Material and Methods

From a stock of *Planaria maculata* originally collected at Woods Hole, Massachusetts and kept in the laboratory for three months, 204 individuals were selected for experimentation. They were divided into two groups of large and small specimens; those of the large size group (Group *L*) measuring 9–12 mm., those of the small size group (Group *S*) measuring 5–8 mm. in length. The animals of each of the groups were distributed into twelve sets—those of Group *L* containing eight individuals each, and those of Group *S* nine individuals each.

The sets were kept in finger-bowls containing 100 cc. of tap water, and were placed on the following diets:

1. Liver, fresh gland.
2. Liver, aqueous extract.
3 and 4. Anterior pituitary, fresh gland.
5. Anterior pituitary, aqueous extract.
6 and 7. Thyroid, fresh gland.
8. Thyroid, aqueous extract.
9 and 10. Anterior pituitary + thyroid, fresh gland.
11. Anterior pituitary + thyroid, aqueous extract.

The animals in set 12 were not given any food and set 12 is hereafter called the " starved " set.

Fresh beef liver, pituitary and thyroid glands were obtained weekly at the slaughter house. The first feeding was made within two hours of the obtainment of the material. It was then frozen, so kept for a week during which time it was given to the planarians, and was then replaced by fresh material.

Small pieces of liver and small pieces of thyroid and anterior pituitary, which had been minced, were placed in the dishes and left there for several hours. The food was then removed, the dishes cleaned and fresh tap water added. Feedings were on alternate days and in some experiments daily. In the anterior pituitary + thyroid set the anterior pituitary and thyroid were given on alternate feeding days. The frequent feedings ensured an adequate food supply for the experimental animals.

Another method of treatment was the addition of filtered aqueous tissue extracts to the tap water. Not only were the animals of the aqueous extract sets exposed to the extract but they also ingested some of it as was indicated by the movements of their pharynges.

The thyroid material was tested throughout by treating frog tadpoles with it. Thyroid glands frozen two weeks retained their potency in that tadpoles feeding on this tissue or exposed to aqueous extracts of it of much lower concentration than those used on the planarians showed greatly accelerated metamorphosis.

Using a binocular dissecting microscope the length and width of the animals were measured as they moved on a glass cover-slip mounted over a piece of millimeter cross-section paper. By regulation of the light source the animals could be made to move along the rulings.

Regeneration

Stevens (1901) and others have reported that the eyes in planarians are regenerated more rapidly at an anterior than at a posterior level. It is also known that eyes regenerate more rapidly in a form such as *P. maculata* possessing high regenerative potentialities than in *Procotyla fluviatilis* possessing, in general, low regenerative potentialities.

In the work which follows the time for eye appearance after decapitation was used as an index of the regeneration rate under the influence of different diets. Following each operation the ventral and dorsal aspects of the animals were carefully examined for the presence of eye pigment.

The anterior portions of all animals were removed five times. The specific data of each decapitation are given below.

Group I

Decapitation No. 1.—Thirty-six days after their first feeding the animals were measured. Two days later four animals from each dish were decapitated by a transverse cut directly posterior to the auricles (Fig. 1, *a*) and placed in an additional set of finger-bowls.

The operated animals comprise Group I. Those which were not operated upon were left in their original aquaria and comprise Group II.

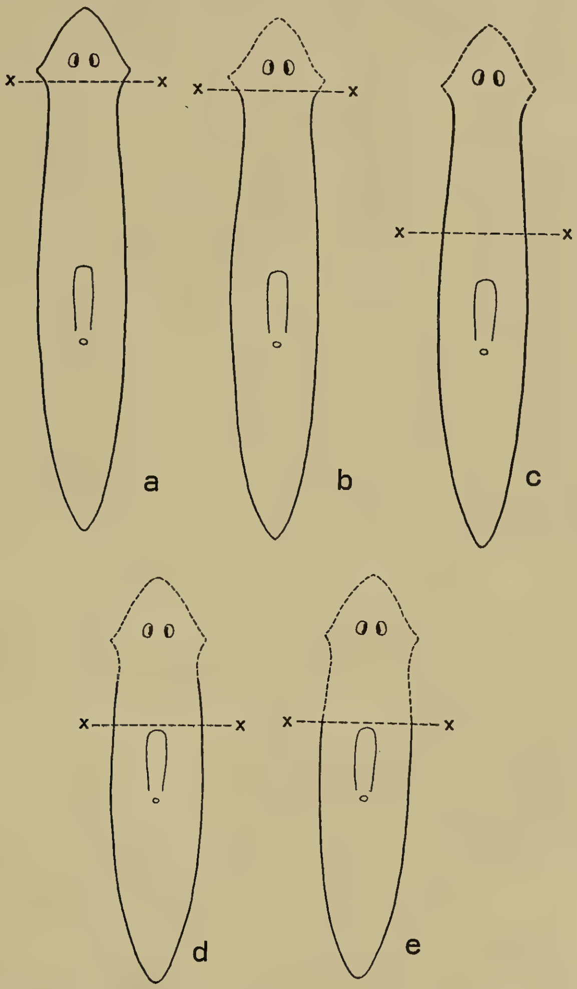

FIG. 1. Decapitations in Group 1. Broken lines indicate tissue regenerated after the previous decapitation. x-x indicates level of cut. All figures diagrammatic. *a,* decapitation No. 1; *b,* decapitation No. 2; *c,* decapitation No. 3; *d,* decapitation No. 4; *e,* decapitation No. 5.

Decapitation No. 2.—Fourteen days after the first decapitation the animals were fed. These feedings were continued during a twenty-four-day period, and the regenerated heads were removed as in decapitation No. 1 (Fig. 1, *b*). Food was placed in the dishes and left there for several hours each day throughout the regeneration period.

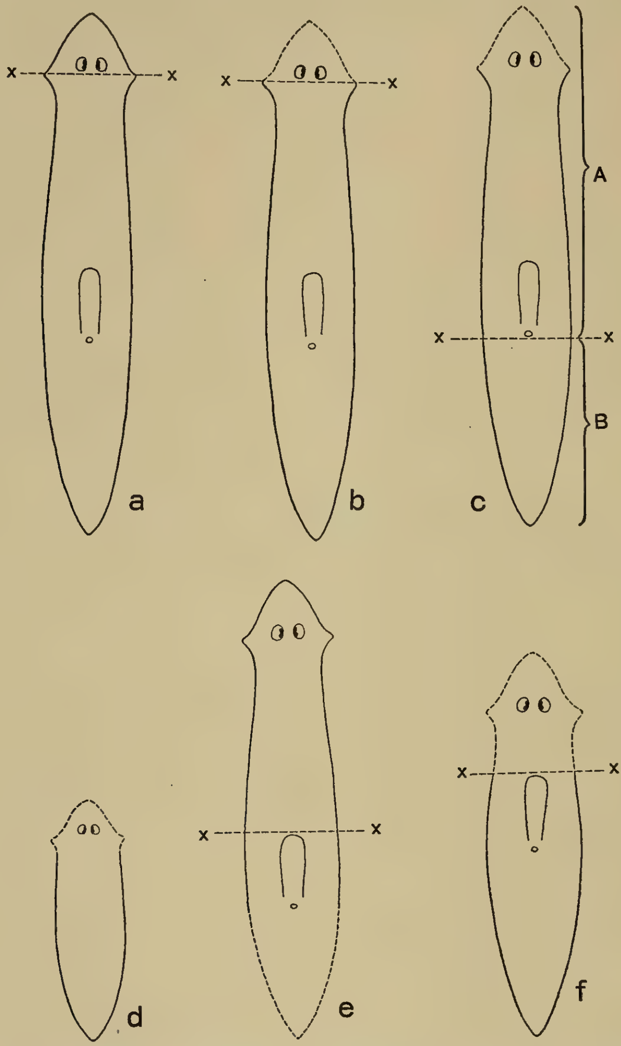

FIG. 2. Decapitations in Group 2. *a,* decapitation No. 1; *b,* decapitation No. 2; *c,* decapitation No. 3 producing *A* and *B* pieces; *d,* portion *B* resulting from decapitation No. 3; *e,* decapitation No. 4 directly anterior to pharynx of portion *A* resulting from decapitation No. 3; *f,* decapitation No. 5.

Decapitation No. 3.—Twenty-one days after the second decapitation the anterior ends were removed by a transverse cut approximately 1 mm. anterior to the pharynx (Fig. 1, *c*). As above, food was placed in the dishes throughout the regeneration period.

Decapitation No. 4.—Twenty-five days after the third decapitation the entire prepharyngeal region was removed (Fig. 1, *d*).

Decapitation No. 5.—The animals were starved for nine days after the fourth decapitation and then all were placed on a liver diet for thirty-five days. Two days after the last feeding the entire prepharyngeal region was removed (Fig. 1, *e*).

Group II

Decapitation No. 1.—Sixty-three days after their first feeding those animals which had not been decapitated previously (Group II) were measured and on the following day were decapitated by a transverse cut directly posterior to the eyes (Fig. 2, *a*).

Decapitation No. 2.—Three days after the first decapitation feeding was recommenced and continued for fifteen days. On the following day the heads were again removed by a cut similar to the first (Fig. 2, *b*).

Decapitation No. 3.—Feeding was begun the day after the second decapitation and continued for nineteen days. The following day a transverse cut was made directly posterior to the pharyngeal pore (Fig. 2, *c*). The posterior portions of the worms (*B*, Fig. 2, *c*) were placed in additional dishes and were observed for eye and head regeneration (Fig. 2, *d*). The anterior portions (*A*, Fig. 2, *c*) possessing the pharynges were fed.

Decapitation No. 4.—Twenty-three days after the third decapitation a transverse cut was made directly anterior to the pharynx (Fig. 2, *e*) in the anterior pieces arising as a result of the previous decapitation (*A*, Fig. 2, *c*).

Decapitation No. 5.—During the eight days following the previous decapitation the animals were not fed. Then they were fed liver on alternate days during a thirty-six-day period. Two days after the last feeding the entire prepharyngeal region was removed by a transverse cut directly anterior to the pharynx (Fig. 2, *f*).

The regeneration rate following the first four operations appeared not to be influenced by the type of food fed if the quantity were sufficient. Following each decapitation the rate of regeneration of a number of the animals varied slightly. The variation in rate within a set of worms on the same diet was as great or greater than the variation among the individuals on different diets (exception noted below).

At the time of the fourth decapitation, 124 days after the first treatment, the animals were in great part composed of tissues built from materials supplied by the particular food to which they were restricted. Notwithstanding this, the results did not differ from those of the previous decapitations.

The exception mentioned above is that of the Liver *L* (gland) set in Group I. Following each of the four operations several worms in this group regenerated more slowly than did any in the other gland sets. In order to ascertain whether this difference in rate was to be attributed to the difference in diet or to an innate tendency of these particular individuals, all of the experimental animals were starved for eight days and then fed only liver for thirty-six days. The anterior ends were then removed (fifth decapitation). Several planarians in the original liver-fed set in Group I, *L,* still regenerated more slowly than the others. Since these animals still lagged behind the others when all individuals in all sets were on the same liver diet, and since the animals in the Liver *L* and Liver *S* (gland) sets in Groups II and those in the Liver *S* (gland) set in Group I did not lag following any of the decapitations, it would appear that the retarded rate was not to be regarded as an indication that thyroid or anterior pituitary glands accelerated or that liver retarded the rate of regeneration. Rather, it would seem that the slowly regenerating animals did so because of innate individual differences.

In Group II, *S,* the animals starved for sixty-four days regenerated more slowly than any of the others. At a temperature of 21° C. eyes made their first appearance as listed:

Solid foods, 140 hours
Aqueous extracts, 160 hours
Starved, 190 hours

In Group II, *L,* the animals starved for sixty-four days regenerated at about the same rate as the others, 142 hours. This may have been due to the larger animals having more of a food reserve.

In Group I, *S,* following the first decapitation thirty-six days after treatment was begun the rate was the same in all groups. Following the second decapitation, at which time the animals had been treated for seventy-five days, the individuals given aqueous extract lagged about twenty hours behind the animals maintained on the fresh gland. The starved forms lagged slightly behind the " extract " forms.

The starved animals and the extract-treated ones which also showed starvation symptoms were fixed and preserved. Those in Group I (*L* and S) were fixed after regeneration had been observed after their second decapitation and those in Group II (*L* and *S*) after their first decapitation.

Retardation after starvation is well known and has been described in planarians by Morgan and in *Triton cristatus* by Morgulis (Morgulis, 1923).

GROWTH

Measurements made as described in the earlier part of this paper are recorded in Table I.

TABLE I

The effects of feeding liver and thyroid glands and of starvation upon the growth of Planaria maculata

Food	A Beginning 2/19		B 36 days after A 3/27		C * 27 days after B 4/23	
	Average length	Average width	Average length	Average width	Average length	Average width
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Liver Gland *S*................	7.7	1.1(9) †	10.4	1.2(9)		
			10.2	1.2(5)	12.1	1.8(5)
Liver Gland *L*................	10.3	1.4(8)	13.8	1.6(8)		
			13.5	1.6(4)	15.5	1.9(4)
Thyroid Gland *S*............	8.0	1.0(17)	8.9	1.1(17)		
			8.8	1.1(9)	9.8	1.4(9)
Thyroid Gland *L*............	10.0	1.4(16)	11.6	1.3(16)		
			11.4	1.3(8)	11.6	1.5(8)
Starve *S*.....................	7.7	1.1(9)	6.1	0.6(9)		
			5.7	0.6(5)	3.8	0.4(5)
Starve *L*.....................	10.7	1.6(8)	8.9	0.9(7)		
			8.6	0.9(3)	6.6	0.7(3)

* A number of animals in each set were decapitated on 3/29. The unoperated animals were again measured on 4/23.

† Figures in parenthesis indicate number of animals in set.

It is clear that the liver-fed animals show the greatest increase, and that thyroid gland, while not causing as much of an increase as liver, certainly does not cause the animals to become smaller than starved individuals as is the case in *P. velata* (Castle, 1928).

A number of worms newly emerged from their capsules were also found to increase in length and width when given only fresh thyroid gland for periods ranging from 15–65 days.

Further evidence that *P. maculata* does not respond to thyroid gland treatment as does *P. velata* is presented by those animals which were on an exclusive thyroid diet for 124 days. During this period their anterior ends had been removed four times with typical regeneration resulting. The animals were in excellent condition, and almost indistinguishable from the liver-fed worms. The only noticeable difference was that the latter were slightly thicker dorso-ventrally.

These results are in agreement with the data presented by Wulzen (Table II, p. 178, 1923).

EGG CAPSULE PRODUCTION

During the latter part of March, egg capsules appeared in some of the dishes. Records of the numbers produced are presented in Table II.

TABLE II

Number of egg capsules produced by Planaria maculata on indicated diets

Number of animals	Food	Number of capsules to 4/22*	Number of capsules to 5/12
9	Liver gland	68	87
18	Anterior pituitary gland	36	40
17	Anterior pituitary-thyroid gland	55	62
18	Thyroid gland	18	20
4	Liver aqueous extract *L*	1	1
5	Liver aqueous extract *S*	0	0
9	Anterior pituitary aqueous extract	0	0
9	Anterior pituitary-thyroid aqueous extract	0	0
9	Thyroid aqueous extract	0	0
9	Starve	0	0

* The animals were decapitated on 4/23. They continued to produce capsules to 5/12.

Comparison of the extremes of the series, the liver-fed with the starved and the extract-treated forms, leads one to believe that low capsule production may be due to a food deficiency. Of the starved and extract-treated forms which were becoming smaller, only Liver Aqueous Extract *L* was productive—a single capsule was produced. The animals in this set were slightly larger than the starved and other extract-treated animals, and smaller than any of those which were fed fresh glands.

The thyroid-fed animals, which were least productive of the gland-fed animals, were in good condition and increasing in size but were smaller than those on the liver diet. Although the planarians fed anterior pituitary and anterior pituitary-thyroid glands approximated the liver-fed ones in all dimensions, they produced a smaller number of egg capsules than the liver-fed individuals.

It is realized that the evidence is too scanty to permit a definite conclusion. It is interesting to note that Greenberg and Schmidt (1936) described an ether-soluble factor in liver which acts as a growth-promoting agent for *Planaria maculata*. Smith and Seegers (1934) found a principle in liver which acts as a growth-promoting agent and which is, in some way, concerned with the typical functioning of the reproductive mechanism in the albino rat.

The writer is indebted to Professor Robert Chambers for his criticism of the manuscript and to Professor F. Gudernatsch for his counsel throughout the course of this work.

SUMMARY

1. Individuals of *Planaria maculata* were fed on abundant, exclusive diets of beef liver, anterior lobe of the pituitary and thyroid glands. Others were given aqueous extracts of the glands or were completely starved.

2. No significant differences were noted in the head regeneration time of the gland-fed animals following each of five amputations of the anterior region.

3. Decapitated animals which were starved and those which were kept on the aqueous extract diet and which showed starvation symptoms regenerated more slowly than those which were fed the fresh glands. There appeared to be a correlation between the rate of regeneration and the initial size of the animals.

4. Thyroid-fed individuals increased in size but to a lesser extent than the liver and pituitary-fed forms. All were in excellent condition following four decapitations. Individuals newly emerged from capsules also increased in size when fed thyroid gland exclusively.

5. Liver-fed animals produced a greater number of egg capsules than any of the others. The starved and extract-treated forms, with the exception of a single capsule by the Liver *L* Aqueous Extract set, produced no capsules.

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RESPONSES OF MUSCLES OF THE SQUID TO REPETITIVE STIMULATION OF THE GIANT NERVE FIBERS

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INTRODUCTION

Investigation of the response of muscles to repetitive stimulation of their nerve fibers has shown that there are great variations among different muscles and different animals in the possibilities of facilitation at the neuromuscular junction. Although the response of an intact muscle fiber, normally activated, is probably always maximal (all-or-nothing), yet in some muscles, for instance those of Crustacea, increase in the frequency of stimulation often increases the tension developed on account of the fact that some muscle fibers are not activated by single, or even by few impulses. On the other hand, in vertebrate striped muscle, unless drugged or fatigued, a single nerve impulse excites all the muscle fibers which it reaches (Lucas, 1909; Adrian and Lucas, 1912).

The range of muscles which have been investigated from this point of view is still small, especially among invertebrates, and we have accordingly investigated the muscular response to repetitive stimulation of the giant axons in the stellar nerves of the squid, *Loligo pealii.* These fibers have been shown to innervate the circular muscle fibers of the mantle, and a single condenser discharge, unless of great intensity or duration, sets up in the axon a single impulse which is capable of activating all of the muscle fibers which it reaches (Young, 1937). The great stellar nerve, containing a single giant axon, was prepared for stimulation in the manner described elsewhere (Young, 1937), and the contractions of a portion of the muscles which it innervates were recorded by attachment to a semi-isometric lever.

Repetitive stimulation was applied by condenser discharges through a thyratron circuit. With the intensity constant and supraliminal the frequency was varied up to approximately seventy stimuli per second. Fatigue set in very rapidly at higher frequencies. Sixteen experiments in which the giant fiber of the great stellar nerve was stimulated yielded consistent results.

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[1] Assisted by a Fellowship of the Rockefeller Foundation.

RESULTS

A typical experiment is shown in Fig. 1. Development of tension is registered by a downward deflection in these records. The first stimulation was at 13 stimuli per second and the second at 24 per second. Incomplete relaxation between twitches occurred in both, and the maximum tension at 24 per second was very slightly greater than at 13.

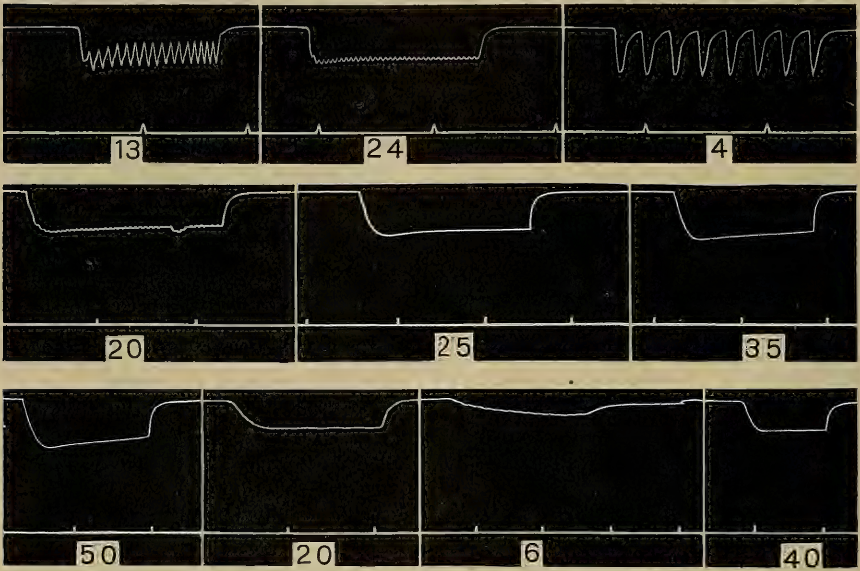

FIG. 1. Record of an experiment in which the giant fiber of the great stellar nerve was stimulated repetitively and the contraction of the mantle muscle was recorded semi-isometrically. Records in order in which they were taken. Numbers indicate frequencies of stimulation.

With stimulation at 4 per second there was complete relaxation between stimuli. In general, complete relaxation between twitches accompanies frequencies of stimulation up to approximately 8 per second. The preparation was then stimulated at 20 and at 25 per second, and showed very slightly increased tension. At 35 stimulations per second fusion of contraction was complete. At 50 per second, as at 35, there was no increase in maximum tension above that at 25 but at 50 the tension declined rapidly, indicating fatigue. The effects of this fatigue were shown when stimulation was returned to 20 per second, where a lower tension was recorded. Thereafter (6 and 40 stimuli per second) the tension varied with the frequency.

Figure 2 shows similar results in three other preparations. In each of these experiments, as in most of the others, there was a slight increase in tension (5–10 per cent) as the frequency increased during the range of incomplete relaxation, an effect which may be ascribed to the mechanical properties of the muscle.

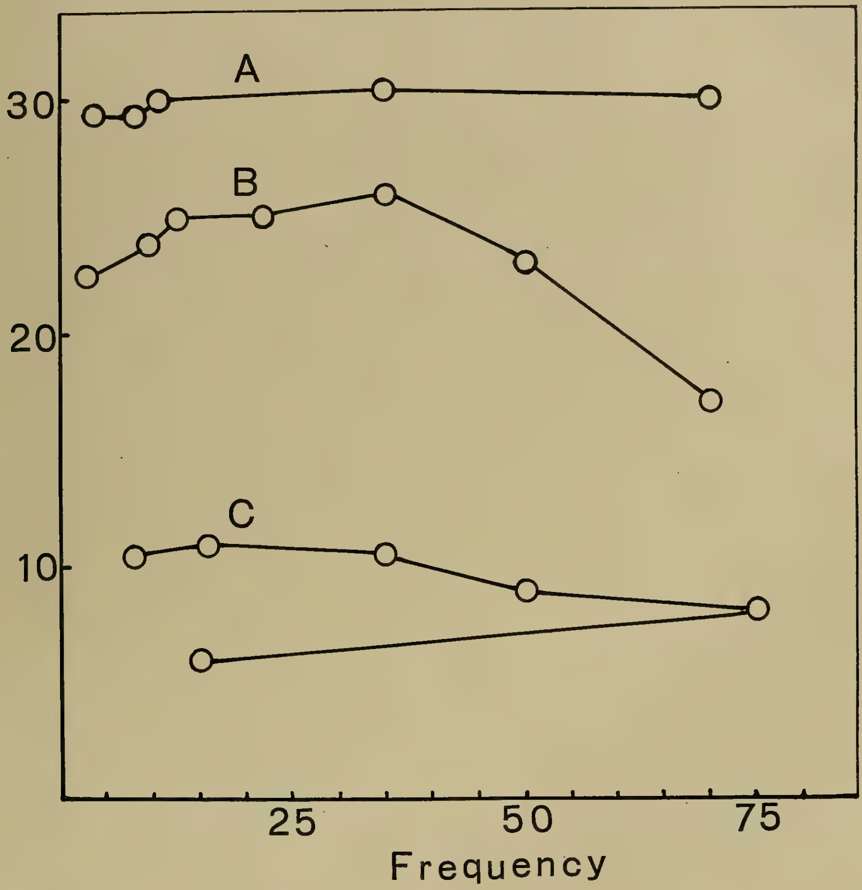

Fig. 2. Plots of maximum tension developed by the mantle muscle against frequency of stimulation of the giant fiber in the great stellar nerve in three preparations. Stimulation proceeded from low to high frequencies. In *C* the lowest point (15 per second) represents response after fatiguing at highest frequency (75 per second).

In Experiment *A*, Fig. 2, the periods of stimulation were brief and there was no increase in tension with increasing frequency of stimulation; this is the general result when no fatigue occurs.

Preparation *B* showed fatigue at 33 stimulations per second, and Preparation *C* at 75 per second. Thereafter the tension fell off and varied with the frequency of stimulation, the higher frequencies eliciting a greater response than the lower.

The preparation is extremely sensitive to strong excitation and high frequency stimulation during one second causes irreversible fatigue. The failure of the response is parallelled by a growing opacity of the mantle.

Discussion

It is evident that there is normally no facilitation at the junction between the endings of the giant fiber and the muscles. Increased frequency of stimulation produces no increase in tension, indicating that all the muscle fibers are activated by each single impulse which reaches them. Young (1937) observed similar results with increasing intensity of stimulation. In the state of fatigue, however, changes occur, probably at some stage in the contractile mechanism, so that summation occurs and greater tensions are produced at the higher frequencies.

This condition is closely similar to that found in the striped muscles of the frog (see Adrian and Lucas, 1912), but contrasts sharply with that of Crustacea, where a single impulse often elicits no mechanical response (Pantin, 1934, 1936). Thus Katz (1936) found that the tension produced by the flexor muscle of the claw of *Maia* increases nearly ten times when the frequency of stimulation is raised from 50–200 per second. Recent observations by Mr. Grossman in the Physiology Course at the Marine Biological Laboratory indicate that the tension developed by the claw of *Limulus* increases from 25 to 650 grams with a rise of frequency from 1–50 per second.

The absence of such facilitation at the neuromuscular junctions of the giant nerve fiber system of the squid is correlated with the function which the system serves in the animal, namely to produce the contractions by which a jet of water is expelled suddenly from the mantle. Once the contraction has occurred the mantle cavity must enlarge again before further work can be done, and there would be no use for sustained or gradually increasing tensions. The expulsion of each jet of water is a single unitary act, performed in an all-or-nothing manner, and any gradation in speed or distance of propulsion must be obtained by variation in the number of contractions set up.

Summary

With increasing frequency of stimulation of a giant nerve fiber in the squid, *Loligo pealii,* the only increase in the tension developed by the circular muscle fibers of the mantle is a small amount (5 to 10 per cent) over the range of incomplete relaxation. The absence of any increased

response at higher frequencies shows that in the fresh muscle a single nerve impulse is capable of activating every muscle fiber which it reaches.

However, the isolated muscle very readily becomes fatigued when stimulated at high frequency and thereafter greater tension is produced at the higher rates. In the normal animal there would be no use for peripheral facilitation and each contraction of the mantle is produced as an all-or-nothing response.

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THE OCCURRENCE OF SAPROPHYTIC FUNGI IN MARINE MUDS

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INTRODUCTION

Since it has been recognized for many years that fungi play an important and significant rôle in the disintegration of organic materials in land soils, it is natural to suspect that they might perform a similar function in sea bottoms. Previous papers (Petersen, 1905; Sparrow, 1934, 1936) have shown that in the littoral of certain localities in northern Europe and eastern United States there are true marine fungi which are active in initiating the destruction of living, autophytic marine plants and in certain cases also in aiding in their disintegration. As no systematic study of off-shore localities for the presence of wholly saprophytic fungi in the muds had been reported, the preliminary investigation described in this paper was undertaken during July–August, 1936, at the Woods Hole Oceanographic Institution.

STATIONS

The following are the locations of stations selected for study and from which the mud cores were obtained. All were in localities marked " sticky " or " sand and mud " on the hydrographic charts.

Station 1. One-half mile N.W. of Weepecket Rock Buoy, Buzzard's Bay. *Depth:* 18.0 meters.
Station 2. Western entrance to Vineyard Sound, 3¼ miles E. of Vineyard Sound Whistling Buoy, 5¼ miles W. by S., ¾ S. on Gay Head Light. *Depth:* 32.7 meters.
Station 3. Gulf of Maine, 45°35′ N., 69°11′ W. (Atlantis Station No. 2640). *Depth:* 163.6 meters.
Station 4. Gulf of Maine, 42°19′ N., 69°20′ W. *Depth:* 220 meters.

Portions of two cores collected by Dr. Henry Stetson from the Gulf of Maine and labelled " Canyon B " (depth 1127.2 meters; Station No. 5, Table I) and " Canyon E " (depth 718.0 meters; Station No. 6, Table

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[1] Contribution No. 73.
[2] Contribution No. 630.

I) were obtained from Dr. S. A. Waksman. Since these had previously been partly used for bacteriological purposes and had been stored for some days they were considered questionable sources of data.

Methods and Results

In order to obtain any significant information in the limited time available, the methods outlined below were considered most practical even though they were subject to very definite limitations.

Collection of Cores

The apparatus commonly used in the collection of stratified mud cores for bacteriological purposes was employed. This is a modification of the instrument used by Moore and Neil (1930) in the Clyde Sea area. To eliminate as far as possible contamination during collection, all parts of the sampling apparatus in contact with the sterilized glass tube were thoroughly swabbed with 10 per cent formalin immediately before use. However, trials with swabbed and unswabbed apparatus showed little difference in the number of colonies of fungi obtained. All the usual precautions were employed to keep the cores free from contamination after collection. The lengths of the cores varied but averaged about 12 cm.; their diameter was 18 mm.

Recovery of Fungi

After the tubes containing the cores were brought into the laboratory, the upper cork was flamed, the free water removed, and the supernatant liquid immediately above the mud put in a sterile container for future use. The core was then " blown " under sterile conditions into a sterilized Petri dish and the outside surface and ends removed with a hot scalpel as an additional precaution against contamination during collection.

Two methods were followed in recovering the fungi. The first, practised extensively in the study of fresh-water fungi, involves the use of water cultures. Five jars of sterilized sea water were prepared into each of which was placed a core from Station 2, a site at which cores were readily obtainable. The cores were broken up in the water, the mixture allowed to settle, and 20–30 dead *Calanus finmarchicus* and *Saggita* from Station 2 were added. Three jars were kept at room temperature (23° C.) in the light and two in the dark at about 6° C. Bits of " shiner " (a small marine fish) removed aseptically were also dropped into the cultures. After 10 days no fungi were found on any of this " bait."

All the significant data were obtained by the plate method. The medium used was the following:

Dextrose	10.0 grams
Peptone	2.0 grams
Agar	15.0 grams
Sea water	1000.0 cc.

This gave a reaction of pH 6.1.

In plating out, samples were taken from the supernatant water, the surface, the middle, and the bottom of the core. The water was distributed, undiluted, among five dishes and the medium added. Five samples were taken from each of the three regions of the core. These were mixed with 1 cc. of sterile water and plated out in the usual manner. Each of the mud samples was about the size of a pea and weighed 200–250.0 mg. In many cases pieces of mud were also laid on the solidified medium, but these dishes merely told whether or not fungi were present on or near the face of the sample. Flasks of unsolidified media were also tried but these became too heavily overgrown with bacteria and protozoans to be of value. As controls for each core, five plates of media were used to each of which was added the contents of a 1 cc. sterile water blank. While these were the actual controls, it will be seen that the ten dishes containing material from the middle and bottom of the core also acted as checks against laboratory contamination.

Table I gives the results of the plating out of the mud samples.

The fungi recovered from the muds by the methods outlined all belonged to genera commonly found in land soils and easily recovered in spore form from the atmosphere. A large majority were species of *Penicillium,* while others belonged to such genera as *Cephalosporium, Trichoderma, Aspergillus, Chætomium, Alternaria, Cladosporium,* and even *Rhizopus.* It was soon evident that no fungi which could be considered characteristically marine were being recovered by these methods and interest in them, qualitatively at least, was greatly diminished.

A preliminary experiment was also carried out to determine whether such fungi might be associated with decaying phytoplankton. Using a sterilized net, two sterilized jars were filled with a heavy concentration of phytoplankton from Vineyard Sound. The material was distributed equally between two sterilized battery jars, both of which were then placed under running sea water in the laboratory. By allowing the water to flow very gently into one jar (" A ") the mass of diatoms soon rested on the bottom. Since the control jar (" B ") was subjected to a stronger stream, the material was soon washed out. After four days, during which time the phytoplankton had gradually disintegrated, five 1 cc. samples were plated out from the bottom of each of the jars. Five

TABLE I

Showing the depth and number of cores taken from the stations as well as the number of samples used, their origin and the number of colonies of fungi recovered.

Origin of samples			Water above core		Surface of core		Surface of core + 1 cc. H_2O blank		Middle of core + 1 cc. H_2O blank		Bottom of core + 1 cc. H_2O blank		Control dishes	
Station	Depth	No. of cores	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of samples	Colonies of fungi	No. of dishes	Colonies of fungi
	meters													
1	18.0	3	15	22	5	1	15	49	15	0	15	1	15	1
2	32.7	4	20	12	15	20	20	59	20	1	20	1	20	1
3	163.6	2	10	17[1]	10	16	10	22	10	0	10	0	10	0
4	220.0	1[2]	5	8[3]	—[4]	—	10	11	10	0	10	1	10	0
5	1127.2	1	5	0	—	—	5	0	5	0	5	1	5	0
6	718.0	1	5	0	—	—	5	2	5	3	5	0	5	0
Totals.......		12	60	59	30	37	65	143	65	4	65	4	65	2

[1] Water very muddy and probably represents fungi from the top layer as well as in water above it.
[2] Two cores were collected but one was discarded because of the possibility that contamination had occurred.
[3] Two of these colonies were at the edge of the dish and were probably contaminations.
[4] There was not sufficient material from Stations 4–6 to use pieces of surface mud alone.

dishes of medium alone acted as controls. Sixteen colonies of fungi were recovered from jar "A" which contained the phytoplankton, and none from jar "B" which contained only the running water. The control plates remained sterile. It was apparent in this case, at least, that there existed a definite relationship between the presence of disintegrating phytoplankton and the moulds. Again, as in the muds, the fungi recovered were all common dust-borne species.

DISCUSSION

From the foregoing, which it must be emphasized is a very preliminary investigation intended more to stimulate further inquiry than to arrive at definite conclusions, certain points seem worthy of further consideration.

If truly marine fungi of a filamentous type exist in the marine muds studied and if there is nothing radically wrong with the methods and type of medium employed, then these organisms must be extremely rare. The types of fungi recovered, i.e., species of *Aspergillus, Penicillium, Trichoderma,* etc., have cast doubts on their being concerned to any great extent in disintegrating processes in the sea. Proof of their presence in marine muds does not mean that they are in an active vegetative state and hence, a working factor in the cycle of decomposition. To the writer's mind, large numbers of direct microscopic observations of mycelium in mud seem the only positive method of demonstrating this important point. The failure of the water cultures to produce saprophytic fungi is also in line with the negative aspects of the data. However, the scanty evidence afforded by the plates of disintegrating phytoplankton presents a more positive picture of relationship. Furthermore, in direct microscopic observations on this disintegrating algal material where freedom from opaque, inorganic matter greatly facilitated the search, active septate mycelium was found in several instances.

Since only land and dust-borne fungi have been recovered it is natural to ask whether they are only contaminants or whether they are actually in the mud. In the collection of the cores every precaution against outside contamination possible with the apparatus was employed and before using the cores the mud surfaces in contact with the tube and the water were cut away. Contamination during culture procedure does not seem likely since mud from the middle and bottom of the cores yielded under identical laboratory manipulation a total of eight colonies in 130 samples and the 65 control plates two. When we consider that 239 colonies developed in the 155 dishes containing samples of supernatant water or mud from the top layer of the core it would seem fairly conclusive that the fungi recovered were actually present in the mud.

Two methods whereby the moulds may have reached the muds are suggested. Either they have been washed from land soils into the sea, or they have been blown, in spore form, from land onto the surface of the water where they have gradually sunk to the bottom. While the stations studied were not in the littoral zone, none was sufficiently offshore to be free from the possibility of obtaining spores from sedimentary material of land origin. Spores are known to be everywhere in the lower atmosphere, and in the immediate vicinity of a continent would be particularly abundant. Many of these would eventually reach the surface of the sea where they might ultimately sink to the bottom. In this connection, qualitative studies (unpublished) by Miss Lois Lillick of the phytoplankton of Vineyard Sound and the Gulf of Maine show that in the former locality fungous spores occur generally throughout the year in the samples, and that they also occur in the shallower waters of the Gulf of Maine, particularly during April and May. It is entirely possible, therefore, that either or both methods of conveyance have contributed fungous material to the marine muds.

Perhaps the most interesting feature of the present investigation has been the information added to our knowledge of the vertical distribution of these ubiquitous moulds. Many types have been recovered from the atmosphere both over land masses and the ocean and even in the stratosphere. Land soils and fresh water have yielded fungi and now they have apparently been recovered in some viable form from the surface of the ocean floor at depths up to 220 meters. Such hardihood is not surprising to those who have observed the capacity of certain of these fungi to withstand adverse environmental circumstances and even to produce under these conditions active vegetative growth.

Finally, it might be emphasized that while no typically marine fungi were found there is strong evidence for believing that certain soil and dust-borne fungi can exist in the surface muds although it has not been shown in what form they occur or that they take an active part there in the disintegration of organic materials.

Summary

Stratified samples of marine bottom were collected under as sterile conditions as possible from four stations which varied from 18 to 220 meters in depth. These stations were located in Buzzard's Bay, Vineyard Sound, and the Gulf of Maine and varied considerably in their distances from land. Attempts were made to recover saprophytic fungi from these cores. Two methods were used: (1) water cultures "baited" with suitable material of marine origin; and (2) the plating out of samples from (*a*) the water immediately above the surface of

the mud core, (*b*) the surface of the core, (*c*) the middle, and (*d*) the bottom of the core. Suitable controls were maintained. No fungi were found in the water cultures. By the plate method using a nutrient medium made up in sea water, 239 colonies were formed in dishes containing water from just above the surface of the core and from the surface of the core itself, eight colonies, in dishes containing material from the middle and bottom of the core, and two colonies in the controls.

A preliminary experiment to determine whether or not fungi were associated with decaying phytoplankton showed definitely that such was the case.

The fungi obtained by the plate method were all common dust and wind-borne forms. Since the methods used in the recovery of fungi did not show in what form they existed on the sea-bottom and since no species which might be called typically marine were recovered, it is doubtful in the present state of our knowledge whether these organisms play an active part in the disintegration of organic materials present in the mud.

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DETERMINATION OF POLARITY BY CENTRIFUGING EGGS OF FUCUS FURCATUS[1]

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INTRODUCTION

The visible inclusions in the protoplasm of many animal eggs have been segregated within the cell into zones or strata by means of the centrifuge. In smaller, less yolky eggs in which the protoplasm is quite fluid, the inclusions, such as yolk and oil droplets, pigment granules, mitochondria (*Arbacia,* Harvey, 1936) etc., segregate in the protoplasm in accordance with their relative densities. In other cases there is sometimes a rotation or dislocation of regions of the cytoplasm which move as a whole.

The results of numerous earlier experiments on animal eggs are reviewed in such standard texts as Morgan's " Experimental Embryology " (1927) and Wilson's " The Cell in Development and Heredity " (1925), and therefore no exhaustive review of the literature will be undertaken here. It should be noted, however, that in general the mere displacement of the visible inclusions has had surprisingly little effect on the development of most of the eggs. The primary polar axis is not determined by the axis of stratification, although Runnström (1927) has shown that dorso-ventrality may be determined in the sea urchin egg. The general conclusion has been that primary polarity depends on factors in the transparent hyaloplasm rather than upon particles large enough to be moved readily by the centrifugal forces employed.[2] By centrifuging unfertilized *Urechis* eggs at 4,800 × g. for very long periods (up to 18 hours), Taylor (1931) was able to determine or shift the primary polar axis considerably, as shown statistically, but it did not come to coincide precisely with the axis of stratification in most of the eggs. Morgan and Tyler (1935) found that when fertilized *Urechis* eggs are centrifuged, the polar bodies may

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[1] This work has been supported in part by funds granted by the Rockefeller Foundation.

[2] Particles moved by the ordinary type of centrifuge may have very pronounced physiological effects, however. Shapiro (1935) has shown that fragments of centrifuged *Arbacia* eggs which contain the heavier granules respire at nearly twice the rate of transparent fragments, and Navez and Harvey (1935) have found twice the indophenol oxidase activity in the fragments containing these granules.

be set free in regions determined by the axis of centrifugation, but in this case the developmental axes of the eggs do not shift with the polar bodies, but instead remain unaltered.

Conklin (1931) found that a dislocation of organs takes place in ascidian eggs when whole regions of the cytoplasm have been displaced or dislocated by centrifuging. Other alterations of the normal development of animal eggs have been brought about by the mechanical consequences of centrifuging, such as twinning (Tyler, 1930; Harvey, 1933, 1935 etc.).

Eggs of the marine brown algæ belonging to the Fucaceæ are more labile than most animal eggs. A number of environmental agents may determine polarity in the *Fucus* egg, including unilateral light (Rosenvinge, 1889) (Kniep, 1907) (Hurd, 1920), especially in the short end of the visible spectrum (Hurd, 1920), direct electric current (Lund, 1923), the presence of neighboring eggs (Rosenvinge, 1889) (Kniep, 1907) (Hurd, 1920) (Whitaker, 1931), especially in acidified sea water (Whitaker, 1937), a pH gradient (Whitaker, 1935) and a temperature gradient (Lowrance, 1937). Knapp (1931) believes that the entrance point of the sperm determines the point of rhizoid origin and the polarity in the egg of *Cystosira,* but the effect of such environmental agents as directed light, if applied at an appropriate time, will supplant this determination and establish a new polarity. The effect of the entrance point of the sperm has not been tested in *Fucus,* but if it determines polarity, this is readily altered by such means as those just indicated. Knapp found further in *Cystosira* that rhizoids form centrifugally when the eggs are centrifuged either before or after fertilization. Schechter (1934) found that rhizoids develop toward the positive pole when pieces of the red alga *Griffithsia* are reared in a direct electric current. Lund (1923) earlier had found that *Fucus* eggs form rhizoids toward the positive pole. Schechter observed that chromatophores migrated toward the positive pole in the *Griffithsia,* and he tried moving the chromatophores by means of the centrifuge (1934, 1935). The chromatophores and other bodies were moved, and accumulated centrifugally under relatively low centrifugal force (150 × g.) so that the cell-materials stratified, but the place of rhizoid origin was unaffected. Polarity was altered, however, in that shoots formed in the regions to which heavier materials had been thrown.

Method and Results

Eggs of *Fucus furcatus* were obtained from the same locality and by methods which have been described previously (Whitaker, 1936).

Experiments were performed in February and March, 1937. Since this species of *Fucus* is hermaphroditic, and sheds egg and antheridial capsules at the same time,[3] fertilization takes place when the egg capsules break down. This can readily be observed, and eggs were used from capsules breaking down during a time-span of 20 minutes or less. The mid-point of this time-span was counted as the average time of fertilization. The eggs were centrifuged at 3,000 × g. An International electric centrifuge was used which tended to warm up during a 20-minute run, and therefore the sea water in the centrifuge tube and the water around the tube were cooled at the start. The temperature of the eggs during the centrifuging rose from about 8° or 10° to 20° C. At all other times the eggs were kept in a humid, dark, constant temperature room at 15 ± ¼° C. After being centrifuged they were briefly exposed only to red light which does not affect the polarity. The pH of the sea water used in the experiments ranged from 7.9 to 8.0, as measured by means of a glass electrode. In order to rule out the effects of neighboring eggs on each other, no egg lying within 5 egg diameters of another was counted in the results (see Whitaker, 1937).

The First Series of Experiments

In the first series of experiments, the eggs were centrifuged in sea water beginning from 12 to 26 minutes after the average time of fertilization, and were then reared in sea water in Petri dishes. Preliminary survey showed that after being centrifuged for 5 minutes the eggs were quite definitely stratified, but not so sharply so as after being centrifuged for 15 to 20 minutes (see Fig. 1, *A*). It was also found, when the eggs were observed about 24 hours later, that the stratified material had redistributed much more markedly if the eggs were centrifuged for only 5 minutes. In all of the experiments to be cited, the eggs were centrifuged at 3,000 × g. for either 15 or 20 minutes (usually 20). Most of the eggs remained spherical and nearly all of those that were distorted by neighbors in the centrifuge tube rounded up again in the Petri dish.

Figure 1, *A* shows a typical egg 23 minutes after being centrifuged for 20 minutes. Three principal zones are sharply demarcated. At the centripetal end, to which the least dense materials are thrown, there is a cap of globules which presumably are oil or fat. Next to this is a dark brown zone in which the chloroplasts are concentrated, and within which the nucleus is concealed. The remainder of the egg, including the centrifugal pole, is essentially transparent, although a few plastids may remain scattered especially in the cortical region, and the general texture appears slightly granular. Figure 1, *B* shows the same egg 15 minutes later, and material of the dark zone is already seen to be moving back toward the center of the egg. Soon afterward the nucleus migrates out of the dark band, moving toward the center of the egg. It is usually closely followed, and sometimes it is flanked along the sides or surrounded, by redistributing dark material. Figures 1 *C* and 1 *D* show other eggs from the same dish at later times. In Fig. 1, *D* it can be seen by referring to the reduced remnant of the oil cap that the rhizoid has formed quite precisely at the centrifugal pole. Some of the eggs

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[3] Most of the antheridial capsules dissolve first so that antherazoids, or sperm, are swimming about the egg capsules when they dissolve and set the eggs free in the sea water.

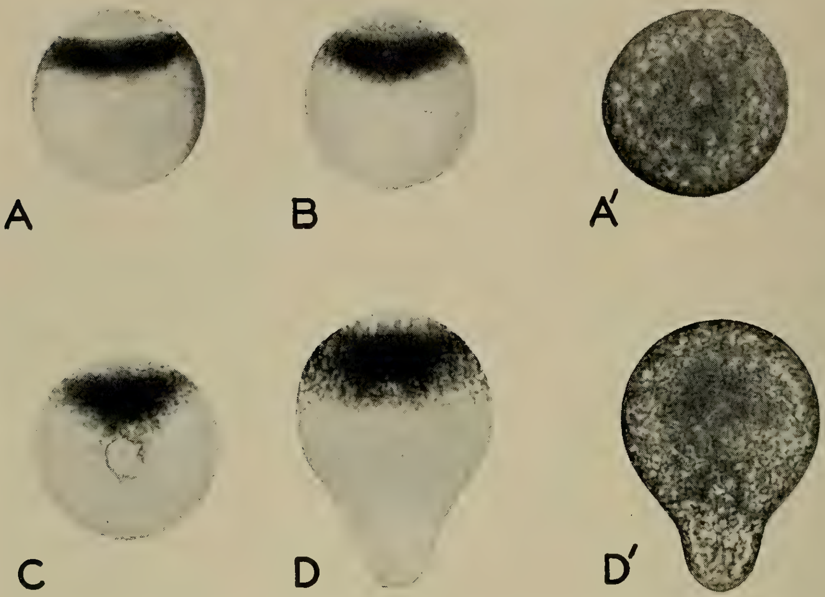

FIG. 1. Photomicrographs of typical developing centrifuged and normal *Fucus* eggs. (*A*) shows an egg 23 minutes after being centrifuged for 20 minutes at 3,000 × g. The centrifuging began shortly after fertilization, and the centrifugal end is below. Chloroplasts are concentrated in the dark centripetal band. (*B*) shows the same egg 15 minutes later, and already inclusions in the dark band are seen to be diffusing back to some extent. (*C*) shows another egg from the same sample 3 hours and 10 minutes later. The nucleus has emerged from the dark stratum within which it was earlier concealed. (*D*) shows another egg from the same sample 13 hours and 25 minutes later. It may be seen, especially by referring to the remnant of the centripetal cap, that the rhizoid protuberance has formed at the centrifugal end of the egg. (*A′*) and (*D′*) are normal non-centrifuged eggs in stages corresponding to (*A*) and (*D*) respectively. All eggs were reared in the dark at 15° C. at pH 7.9–8.0 (see text).

do not remain stratified but redistribute completely. These will be referred to later.

In six similar experiments of the first series, large numbers of eggs were reared in Petri dishes after being removed from the centrifuge, and the final records were taken 20 to 24 hours later. When centrifuged eggs are placed in a dish of sea water, most of them rotate and lie so that the heavy side is down. When the rhizoid extends downward it lifts the cell body which usually rolls over to one side. The strata can then be seen. Each egg was further rolled over by hand with a glass needle if necessary to observe the stratification when the results were recorded.

Sample counts of over 1,000 eggs indicated that the vast majority of the eggs which remained stratified, so that the axis of centrifugation could be identified, formed rhizoids at or near the ends to which heavier materials had been thrown. In three experiments more precise estimates were made of the positions of the rhizoids. A random count of 490 eggs showed that 96 per cent formed rhizoids within 10° of the centrifugal pole, and the remaining 4 per cent formed them between 10° and 22° from the pole. In addition to the eggs which remained stratified (Fig. 1, *D*), and could be analyzed as above, some eggs redistributed the stratified materials so completely that the axis of centrifugation could not be identified. The proportion of these was usually small, about 4 to 10 per cent, although in one set of eggs, for reasons which are not clear, approximately 50 per cent of the eggs had redistributed at 24 hours. There was usually a sharp distinction between eggs that remained clearly stratified and those that had completely redistributed, with relatively few transitional cases.

From the experiments cited above, it was concluded that the rhizoid forms at the end of the egg to which heavier materials are thrown, at least in the eggs which remain stratified after 24 hours. The *Fucus* egg is spherical and has no early identifying mark of polarity (Fig. 1, *A'*). It is conceivable, however, that the egg actually has a predetermined rhizoid pole which is also the heaviest part of the egg. If so, in centrifuging, the egg would be thrown to the bottom of the tube in an oriented position. This does not appear very probable, but if it were true the stratification would conform to the oriented position and might itself be irrelevant in the determination of polarity. A second series of experiments was therefore designed to test this and other points.

THE SECOND SERIES OF EXPERIMENTS

In the second series, eggs were embedded in 1.5 per cent agar-sea water before centrifuging, after centrifuging, and without being centrifuged. Eggs develop normally in this medium, and observations on stratified eggs in various positions indicate that they are firmly held and that few if any move or rotate within the agar-sea water after it has cooled and jelled, if it is undisturbed. If the agar is cut with a razor, eggs lying close to the cut may rotate as a consequence of the mechanical disturbance. One and a half per cent agar-sea water is a firm jelly at 15° C., and the surface of a fertilized *Fucus* egg attaches to it firmly.

Eggs were embedded by pipetting a concentrated mass of eggs in a minimum of sea water into agar-sea water at 38° C. The agar-sea water gelates below this temperature. The mixture was taken in and out of the agar pipette once or twice to assure mixing and the dish was put on ice to cool rapidly and shorten the exposure of the eggs to high temperature.

When a population of fertilized eggs which are not centrifuged is embedded in agar-sea water and reared in the dark, the rhizoids form at random in all directions as seen from above. The upward and downward components are not equal, however, when the population is considered statistically. Rhizoids are found in every possible position, from straight down to straight up, but more are found with a downward component than with an upward component. The average position is somewhat downward from the horizontal plane. For example, in a population of 413 eggs, 14 per cent of the rhizoids formed upward (± 45°), 41 per cent to the side (± 45°), and 45 per cent downward (± 45°). In other populations more rhizoids formed to the side than downward. It has been shown elsewhere (Whitaker, 1937) that the downward component increases with acidity of the sea water and with increased concentration of eggs. It cannot be said at the present time whether the downward component is due to gravity or to concentration gradients of substances (e.g. CO_2) which diffuse through the upper boundary of the medium but cannot diffuse through the bottom of the dish.

When eggs were first centrifuged, and then mixed at random into agar-sea water, it was found by analyzing 600 eggs statistically after the agar had solidified that the eggs tended to be oriented with the heavy side downward. This orientation, while appreciable, was on the whole not pronounced. It presumably took place just before the agar solidified, as a result of the strong density gradients which had been created in the eggs by stratification in the centrifuge. The results from these eggs which bear on the determination of polarity will be considered after the principal results of the second series have been taken up. These are derived from eggs first embedded in agar-sea water and then centrifuged.

In six similar experiments of the second series, eggs were embedded at random soon after fertilization in 1.5 per cent agar-sea water in moulds which formed castings which fitted the bottoms of the centrifuge tubes. The castings were centrifuged, and then small blocks or strips of agar, bearing stratified eggs, were cut with a razor and placed in Petri dishes of sea water. All eggs in a block were oriented the same way with respect to their stratification, except for some eggs, especially those near the edge, which were mechanically disturbed and rolled when the block was cut and handled. These blocks were oriented in three different positions: with the centrifugal side (1) straight down, (2) to the side, and (3) straight up. In each experiment, blocks of agar cut from the same centrifuged piece were placed in either two or three of the positions.

After the eggs in these blocks of agar-sea water had developed in the dark for 20 to 24 hours, they were inspected with the microscope and sample counts were made of the positions of rhizoid origin. Whenever necessary, eggs were rolled over within the agar with a glass needle. The eggs were classified as having formed rhizoids downward within 45°, or to the side within 45°, or upward within 45°. The positions of the rhizoids with respect to stratification were noted, and whether or not redistribution had taken place so that stratification was no longer discernible. The results of the separate experiments were essentially similar, and therefore the averaged results will be considered.

Of 423 eggs in agar-sea water blocks placed so that the centrifugal sides of the blocks were downward, 46 or less than 11 per cent had redistributed, while the remaining 89 per cent remained stratified. Ninety-nine per cent of the eggs which remained stratified formed rhizoids within 10° of the centrifugal pole (see Fig. 1, *D*), while 1 per cent formed them farther away from the centrifugal pole, but still on the centrifugal hemisphere. Ninety-five per cent of the eggs which remained stratified had retained their position in the agar, without rotating after being centrifuged, so that their centrifugal poles were downward, while 5 per cent had been rotated so that their centrifugal poles were to the side within 45°. Of the 46 eggs which had redistributed, 3 formed rhizoids downward, 25 to the side, and 18 upward. The distribution of these rhizoids is strikingly different from those on the eggs which remained stratified. Relatively many more are to the side and upward, which suggests either that the determination of rhizoid formation at the centrifugal poles is largely lost when eggs redistribute, or else that a large proportion of the redistributed eggs had been oriented with their centrifugal poles to the side or upward (as a result of rotating when the block was cut), and that such orientations favor redistribution. Evidence that both of these factors operate is provided by eggs reared with their centrifugal poles upward.

Eggs reared in agar-sea water blocks placed so that the centrifugal sides of the blocks were upward were held with the heaviest materials at the top and the lightest at the bottom. This is the exact opposite of the position which the eggs tend to assume when free in a dish, and it should tend to favor redistribution since the effect of gravity is added to that of diffusion. Of 488 eggs which developed in blocks in this position, 166, or 34 per cent had redistributed (compared with 11 per cent in blocks placed centrifugal side downward), and of these redistributed eggs 130 formed rhizoids downward, 33 to the side, and 3 upward. In other words, the percentage of redistribution was trebled, and the redistributed eggs formed rhizoids more nearly in the general pattern found in a population of eggs which have never been centrifuged. Even more rhizoids formed downward than in the average population of non-centrifuged eggs. Three hundred and twenty-two eggs remained stratified, and 93 per cent of these formed rhizoids within 10° of the centrifugal pole. The remaining 7 per cent formed them farther away from the centrifugal pole, but not more than 90°. Two hundred and seventy-two eggs formed rhizoids upward, and 99 per cent of these had remained stratified and formed rhizoids within 10° of the centrifugal pole. Twenty-six of the 488 eggs remained stratified but had been rotated so that the centrifugal pole was to the side (± 45°), and in 27 it was downward (± 45°).

Eggs reared in agar-sea water blocks placed so that the centrifugal sides of the blocks were to the side gave results which are on the whole intermediate between those from eggs in blocks in up and in down positions. Four hundred and twenty-five eggs were analyzed. Sixteen per cent had redistributed, compared with 34 and 11 per cent, respectively, in the other two positions. These redistributed eggs formed 1 rhizoid upward, 39 to the side, and 27 downward (all ± 45°). Three hundred and fifty-eight eggs remained stratified and 333 of these (93 per cent) formed rhizoids within 10° of the centrifugal pole. The remaining 7 per cent formed them between 10° and 90° from the centrifugal pole. Of the 333 eggs which formed rhizoids within 10° of the centrifugal pole, 314 (94 per cent) developed rhizoids laterally (i.e., they retained their original orientation in the agar blocks).

As earlier mentioned, eggs were also centrifuged first and then embedded in agar-sea water. The axes of stratification lay at various angles with respect to the horizontal plane. The results obtained by analyzing 600 eggs selected at random were entirely in harmony with those just cited.

DISCUSSION

Most of the centrifuged eggs remain stratified long after the rhizoid protuberances have formed, although the stratified materials begin to diffuse back to some extent quite early (see Fig. 1). The results show that, regardless of the position in which they are held and reared, the centrifuged eggs which remain stratified form rhizoids on their centrifugal hemispheres. With very high incidence, they do so quite precisely at their centrifugal poles to which heavier materials have been thrown. Thus, of 1,057 eggs which remained stratified in blocks of agar-sea water placed so that the centrifugal sides were downward, to the side, or upward (comparable numbers of eggs being in each of these 3 positions), 1,007 or more than 95 per cent formed rhizoids within 10° of their centrifugal poles as marked by the strata. The remainder formed them between 10° and 90° from the centrifugal poles. These eggs were embedded in random positions in firm agar-sea water before being centrifuged, and presumably could not orient in the centrifuge in accordance with any pre-determined polarity. The developmental polarity is therefore determined by the axis of centrifugation. If there is an earlier polarity (as found by Knapp (1931) in *Cystosira;* see introduction), it is completely altered.

Some of the eggs do not remain stratified, but instead the visible inclusions have redistributed so completely when they are observed after the rhizoid protuberances have formed that they are indistinguishable from eggs which have never been centrifuged. When eggs are held inverted in agar-sea water so that the centrifugal poles are uppermost, the proportion of the eggs which redistribute is considerably increased, although the redistributed eggs still remain in the minority. When inverted eggs redistribute, the tendency to form rhizoids at their centrifugal poles disappears. They form rhizoids more nearly in the positions in which they would have formed them if they had never been centrifuged. In fact, both in the blocks placed centrifugal side upward and in those placed centrifugal side downward, the redistributed eggs formed even more rhizoids on the sides of the eggs away from the centrifugal sides of the agar blocks than non-centrifuged eggs would have been expected to do in their place.

The fact that only some of the eggs redistribute, while most others held in the same position do not, must be attributed to variations in the properties of different eggs in regard to factors such as viscosity, etc., and perhaps to the fact that some of the eggs had been fertilized as much as 20 minutes longer than others at the time of centrifugation.

Knapp (1931) found that rhizoids form at the centrifugal poles of

Cystosira eggs, in high percentages if the eggs have been visibly stratified. The principal results on *Fucus* eggs are thus similar. Schechter's (1934, 1935) results on pieces of the red alga *Griffithsia* are similar in that the location of organs was determined by centrifuging, but in this case shoots, not rhizoids, formed at the centrifugal pole. The place of origin of rhizoids was unaffected.

It is possible that a special rhizoid-forming substance accumulates at the centrifugal pole of the *Fucus* egg as a result of centrifugation. However, since gradients of a number of factors which affect the rate of activities such as respiration, CO_2 production, etc. (e.g. temperature, pH, etc.; see Introduction) determine the point of rhizoid origin in this egg, it is also possible that polarity is determined in the stratified egg by gradients of such activities caused by the asymmetrical distribution of cell inclusions. Moreover, these factors may influence the mitotic figure which in turn may affect the polarity. The present experiments do not provide a basis for choosing between these possibilities.

SUMMARY

1. When eggs of *Fucus furcatus* are centrifuged at 3,000 × g. for 15 or 20 minutes, beginning between 12 and 37 minutes after fertilization, the visible cell inclusions are thrown centripetally (see Fig. 1).

2. Most of the eggs remain visibly stratified until long after the rhizoid protuberances have formed. Ninety-three to ninety-nine per cent of the eggs which remain stratified form rhizoids quite precisely at the centrifugal pole (within 10°; see Fig. 1), even when they have been embedded in random positions in firm agar-sea water before being centrifuged so that they could not orient in the centrifuge in accordance with any earlier polarity. The remainder also form rhizoids on the centrifugal hemisphere of the egg, but farther removed from the centrifugal pole.

3. In eggs which remain stratified, the place of rhizoid origin and the developmental polarity are therefore determined by the axis of centrifugation. This is true regardless of the position in which the stratified eggs are held in agar-sea water during development. If there is an earlier polarity in the egg it is completely supplanted by the effects of centrifugation.

4. In a smaller proportion of the eggs, the cell inclusions have redistributed so completely when the eggs are inspected after the rhizoid protuberances have formed that, in high contrast to the eggs which remain stratified, they are visibly indistinguishable from eggs which have never been centrifuged. Such redistribution is more prevalent when eggs are reared heavy side up in agar than when they are reared heavy

side down. The determination of rhizoid formation at the centrifugal pole is lost in inverted eggs which have redistributed. Under the conditions of the experiments, the determination therefore appears to correlate with the distribution of visible inclusions.

The author is indebted to Mr. C. W. Clancy for assistance in carrying out the experiments.

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ON THE ENERGETICS OF DIFFERENTIATION, VI

Comparison of the Temperature Coefficients of the Respiratory Rates of Unfertilized and of Fertilizd Eggs [1]

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The results of these experiments show principally that the temperature coefficients of the rates of respiration are the same for unfertilized eggs as for fertilized eggs over most of the temperature range investigated. They diverge somewhat at the lower temperatures, the fertilized eggs giving higher values.

Theoretical Part

An unfertilized egg is generally considered as a resting cell, presumably being concerned merely with keeping itself alive. It should thus exhibit simply maintenance (basal) metabolism. The maintenance metabolism of an organism is measured by the heat production or the respiration under " resting " conditions. The respiration of an unfertilized egg may then be taken as a measure of its maintenance requirements.

A fertilized egg is also considered as having a maintenance requirement, but in addition there are requirements for processes termed growth and differentiation. It is conceivable that by such an agent as change in temperature, these three processes might be affected differently. But from experiments on the effect of temperature on the rate of development and the rate of respiration of fertilized eggs (Tyler, 1936), this does not appear likely, unless these changes are compensatory. The cited experiments had shown that there is the same total oxygen consumption in reaching a given stage of development at one temperature as at another. Thus the temperature coefficients of rate of respiration and development are the same and there is no temperature within the normal range at which development is accomplished with a minimum of respiration.

It was to be expected, then, that the rate of respiration of unfertilized eggs should give the same temperature coefficients as that of de-

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[1] This investigation was supported in part by a grant from the Penrose fund of the American Philosophical Society.

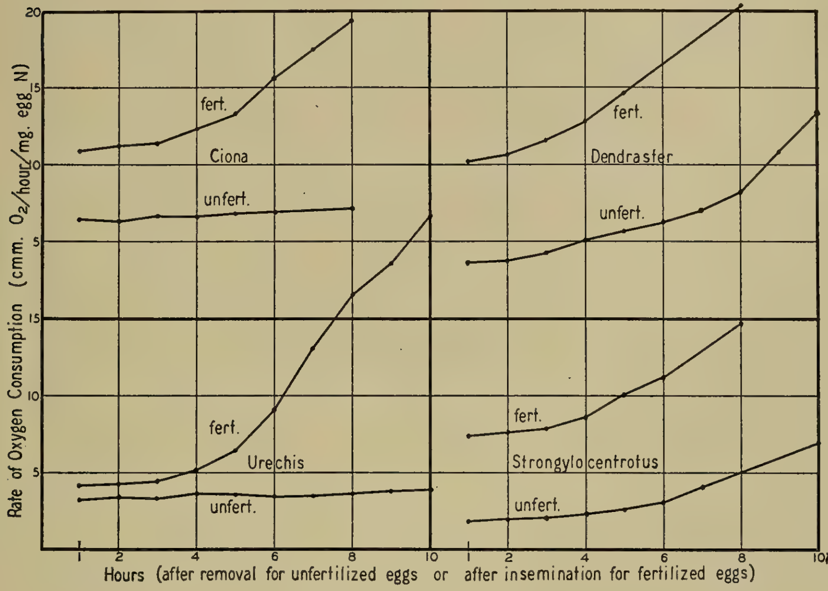
veloping eggs. An investigation of this sort was made by Rubenstein and Gerard (1934) on the sea-urchin egg. They found much higher coefficients for the unfertilized than for the fertilized eggs, the average values for Q_{10} being 4.1 for the former and 1.8 for the latter. An examination of their data shows a number of errors in the calculations which, while not greatly affecting the average values, make their conclusions somewhat less convincing. Considering also the difficulties involved in measuring the respiration of unfertilized eggs, it seemed advisable to repeat these experiments on the sea-urchin and in addition to investigate other forms.

EXPERIMENTAL PART

Material and Methods

The eggs used were those of the sea-urchin, *Strongylocentrotus purpuratus;* the sand-dollar, *Dendraster excentricus;* the echiuroid worm, *Urechis caupo;* and the ascidian, *Ciona intestinalis.*

The manometric method of Warburg was employed for measuring the respiration. Since one of the principal difficulties with unfertilized eggs is their relatively low rate of respiration per unit mass, special vessels were constructed which would tend to make the measured pressure changes as large as possible, at the same time insuring adequate gas exchange. Considering the various factors involved, the cylindrical type of vessel previously described (Tyler, 1936), but with calibration volumes of 18 to 20 cc. and capable of taking 8 cc. of egg suspension, was employed. The vessel constants are of the order of 1.0. The eggs are pipetted into the vessels with special automatic pipettes. Errors in calibration of the vessels and of delivery from the pipettes amount to less than 0.1 and 0.3 per cent and are therefore negligible. The amount of material employed was determined from the nitrogen content of the eggs, obtained by means of a modified Kjeldahl method. The error here depends upon the amount of material employed, but even for the most dilute egg suspension it does not exceed one per cent. In some instances, noted as " no Kjeldahl " in the tables, no nitrogen determinations were made, but the eggs were simply pipetted from a uniform suspension. The manometers are read to the nearest 0.5 mm., so the reading error will be determined by the magnitude of the pressure change. In general no coefficients were calculated for pressure changes of less than 10 mm. and in most cases the readings used were between 30 and 80 mm. In cases in which only dilute suspensions of eggs are available, the runs must be continued over longer periods of time to get sufficient pressure change to reduce the reading error. This involves another difficulty, One of the chief difficulties that was encountered is the variation in rate of respiration during the progress of a run. The rate of respiration of the unfertilized egg does not remain constant, as is usually assumed, but rises after a shorter or longer period of time. This rise has been previously noted by Warburg (1915) and by Runnström (1930). We find that the unfertilized eggs of the different animals we have studied vary in regard to the rate of rise. This is illustrated in Fig. 1. It may be seen that the eggs of *Dendraster* and *Strongylocentrotus* show a much more rapid rise than do the eggs of *Ciona* and *Urechis*. In fact, in *Dendraster* and in *Strongylocentrotus* the unfertilized egg respiration rate rises almost as rapidly as does the fertilized egg respiration rate. This rise in respiratory rate appears to be correlated with the loss of fertilizability of the eggs. Without presenting quantitative data at this time, it may be pointed out that *Ciona* and *Urechis* eggs remain fertilizable after standing 24 to 48 hours in sea water whereas *Dendraster* and *Strongylocentrotus* eggs are no longer fertilizable after 5 to 8 hours. Different batches of eggs differ somewhat in the rate of rise, but the



EXPLANATION OF FIGURES

FIG. 1. Rates of oxygen consumption of unfertilized and fertilized eggs of *Ciona, Urechis, Dendraster* and *Strongylocentrotus*. The unfertilized rates for the two latter rise much more rapidly with time than for the other two.

curves of Fig. 1 are typical. The unfertilized eggs of all the forms investigated show sooner or later a rise in respiratory rate.[2]

Occasionally in some runs (e.g. Experiment XI of Table I) a decrease in rate is manifested in the early part of a run. This appears to be due to the sticking of the unfertilized eggs to the walls of the vessels. In some cases a considerable amount (perhaps 5 per cent) of the eggs stick to the walls. The stuck eggs are presumably unaffected while under the sea water, but when the shaking is stopped for a reading some of the stuck eggs are left well above the water level in the vessels. Such eggs, if partially or completely cytolyzed, would give an abnormal respiratory rate. In our experiments on cytolyzing eggs with dry ice or with distilled water we find an initially high respiration followed after about an hour by a very low rate. If this holds for the stuck eggs we would expect to get slight decreases in rate as well as slight initial increases, since most of the sticking occurs at the start of a run.

Due to the variations in rate of respiration with time the temperature coefficients cannot be determined simply by placing a vessel first at one temperature then at another. It is necessary in addition to run eggs from the same batch simultaneously at the two temperatures. The experiments were therefore performed in the following manner. Usually, four vessels were prepared from the same batch of eggs as soon as possible after removal from the animal and two of them were placed in each of two different temperature baths. After a certain number of readings were made the vessels in the high temperature bath were exchanged with those in the low temperature bath. After another set of readings, the vessels were replaced in the original baths and the readings continued. At least fifteen minutes was allowed for temperature equilibrium to be attained.

The temperature coefficients were then calculated in two ways; first, from the oxygen consumption in the vessels run simultaneously at the two temperatures; secondly, from the oxygen consumption in one vessel run alternately at the different temperatures. For the first type of coefficient it is important to know the quantities of eggs in the different vessels or to have identical samples of a uniform suspension in each. For the coefficients calculated in the second manner this is, of course, unnecessary. Errors in the determinations of the quantity of eggs therefore do not enter into the coefficients calculated in the second manner. However, variations in rate of respiration with time will significantly affect the coefficients calculated in the second manner. To compensate for this the average of two values is taken. For a vessel starting in the high temperature bath, one value is obtained by dividing the initial rate of oxygen consumption by the rate during the ensuing period in the low temperature bath. The other value is gotten by dividing this same low temperature rate into the rate during the following period in the high bath. Where the rate rises, as it generally does, the first value will be lower than the second but the average of the two will be nearer the true value. For a vessel starting in the low temperature bath the two values are obtained in a similar manner. In this case an increasing rate will make the first value too high and the second too low, but again the average will be nearer the true value. The principle involved is the same as in determining the rest point of a balance.

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[2] The rise is not due to conditions in the respiration vessels, as an experiment with *Urechis* illustrates. Two samples of eggs, run continuously for 20 hours at 22°, gave Xo_2's of 3.05 and 3.00 cu. mm. in the first hour, and 5.12 and 5.35 cu. mm. in the twentieth hour. Two more samples of eggs from the same batch, that had stood for twenty hours and washed before using, gave Xo_2's of 4.81 and 5.06 cu. mm. in the first hour. Subsequent fertilization was 85 to 100 per cent. The capacity for fertilization is not lost until after a considerable rise in respiration is manifest. In some experiments 100 per cent fertilization was obtained after an almost two-fold rise in the unfertilized rate.

In the tables, the coefficients calculated in the second manner are not given for each individual vessel, but the average for each pair of temperatures is listed in the line "average for individual vessels." The respiration values from which the coefficients are readily determined are given under the headings X_{O_2}. The probable errors are given in cases in which fifteen or more values are averaged. For this purpose, the average for each vessel, determined as mentioned above, is considered as a single value. The last two columns of the tables give the coefficients determined in the first manner; that is, for oxygen consumption during the same period of time at the two temperatures. Where duplicates are run, the coefficient is the ratio of the average oxygen consumption in the two vessels at each temperature. The mean of these values for each pair of temperatures is given in the line marked "average" in the tables. In determining this average an experiment with only two vessels is weighted one-half (rather than one-quarter); that is, two experiments with two vessels are considered the same as one experiment with four vessels. The probable errors are again given for cases in which fifteen or more values are averaged.

In most of the experiments only unfertilized eggs were run, a considerable number of coefficients for fertilized eggs having been obtained in previous work (Tyler, 1936). These values for fertilized eggs are listed in the lines "average from previously published data" in the tables. In some experiments (e.g. VI, X and XI of Table I) two vessels with fertilized eggs were run along with two vessels with unfertilized eggs from the same batch. The figures in parentheses in the tables are for fertilized eggs. In some experiments (I, V and VI of Table V) after an unfertilized run, the eggs were inseminated in the vessels and the measurements continued. The fertilized eggs give in general much more consistent values, so relatively fewer experiments, in addition to those previously published, were required. The coefficients for the fertilized eggs were calculated here in the same two ways as for the unfertilized eggs. The values taken from previously published data were obtained in a somewhat different manner (*loc. cit.*) which automatically took into account the rising rate of respiration of the fertilized eggs. However, since the rate rises very slowly at the start and since the methods of calculation also allow for the rising rate, comparable values are obtained for short runs. In prolonged runs, values for fertilized eggs obtained in this manner would tend to deviate because the rate of rise increases somewhat with time.

TABLE I

Eggs of Urechis. X_{O_2} = mm.^{3} O_2 consumed per mg. egg nitrogen; figures in parentheses are for fertilized eggs, the rest are for unfertilized eggs. Q_{10} = ratio of X_{O_2} values (average X_{O_2} where duplicates are run) for the same time intervals at the two temperatures.

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	° *C.*			° *C.*				
I	1	22	1.99	1.90	12	0.82	0.83	2.37	
	1	12	0.84	0.91	22	2.09	2.17	2.43	
	1	22	2.43	2.22	12	0.97	0.96	2.41	
II	2	22	6.10	6.00	12	2.30	2.04	2.79	
	2	12	2.54	2.49	22	6.43	6.28	2.53	
	2	22	6.41	6.19	12	2.19	2.04	2.98	
III	2	22	4.35	4.24	12	1.79	1.80	2.39	
	1	12	1.07	1.08	22	2.35	2.30	2.17	
	2	22	4.62	4.32	12	2.02	2.05	2.20	
IV	2	22	4.77	4.60	12	2.02	2.05	2.30	
	2	12	2.34	2.22	22	5.03	5.09	2.22	
	2	22	6.24	6.00	12	2.66	2.54	2.35	
V	1	22	2.51	2.43	12	0.89	0.91	2.75	
	1	12	1.01	0.95	22	2.62	2.59	2.65	
	1	22	2.46	2.48	12	1.00	0.95	2.53	
VI	1	22	1.38	(3.38)	12	0.67	(1.43)	2.06	2.37
	1	12	0.62	(1.32)	22	1.64	(3.38)	2.65	2.56
	1	22	1.64	(3.14)	12	0.87	(1.52)	1.88	2.07

TABLE I (*Continued*)

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	° *C.*			° *C.*				
VII	1	22	3.58	3.35	12	1.28	1.25	2.74	
	3	12	3.87	3.93	22	10.91	11.28	2.85	
	3	22	10.14	10.28	12	3.99	4.17	2.50	
VIII	1	22	4.49		12	1.77		2.54	
	2	12	3.00		22	8.23		2.74	
	3	22	10.87		12	3.35		3.24	
IX	2	22	7.38		12	2.90		2.55	
	2	12	2.58		22	6.27		2.43	
	2	22	6.44		12	2.30		2.80	
X	1	22	4.42	(4.50)	12	1.82	(1.65)	2.43	2.73
	1	12	1.20	(1.70)	22	4.46	(4.75)	3.71	2.79
	1	22	3.69	(4.69)	12	1.82	(1.68)	2.03	2.79
XI	1	22	5.06	(4.75)	12	1.96	(1.62)	2.69	2.93
	1	12	1.55	(1.78)	22	5.12	(4.80)	3.30	2.70
	1	22	4.33	(4.88)	12	1.82	(1.72)	2.39	2.84
Average								2.57 ± 0.03	2.64
Average for Experiments II, V and VII								2.70	
Average for individual vessels								2.57 ± 0.04	2.65
Average for individual vessels, Exp. II, V and VII								2.77	
Average for fertilized eggs from previously published data									2.79 ± 0.02

Samples of the eggs from the vessels were inseminated at the end of each run and gave in most cases 90 to 100 per cent fertilization. The experiments in which it was less are as follows: Table I, I, 85, 75, 90, 90; IV, 50, 55, 85, 85; Table III, II, 70, 85; IX, 80, 70, 90, 85; Table IV, XIV, 45, 75; Table VI, I, 15, 20, 35, 30; II, 10, 10, 50, 20. There is no evident relation between the low fertilization in these cases and values of the corresponding temperature coefficients, as an examination of the tables shows.

It is clear from temperature experiments on biological material that coefficients determined at different parts of the temperature scale are not alike. They generally increase as the temperature range is lowered. In other words, Q_{10} (and even μ of the Arrhenius equation) is not a constant. We must therefore make our comparisons for the same temperature intervals and not use the average of values obtained from all parts of the temperature scale. We have concentrated on one pair of temperatures in attempting to obtain consistent values and supplemented with fewer experiments at other temperatures.

Urechis caupo

Seventeen sets of experiments were run with the eggs of *Urechis;* eleven at the temperatures 22° and 12°, three at 20° and 10°, two at 18° and 8° and one at 15° and 5°. The experiments at 22° and 12° are listed in Table I. The average of the coefficients for the unfertilized eggs is 2.57 by both methods of calculation. For the fertilized eggs (Experiments VI, X and XI) the average Q_{10}'s are 2.64 and 2.65 respectively by the first and by the second methods of calculation. From previous data the value for fertilized eggs is 2.79. Considering the variation in the individual values, we can only conclude that there is no significant difference between the coefficients for the unfertilized and the fertilized eggs in this temperature range.

Comparison of the first and last values of X_{O_2} in each experiment gives the change in rate of respiration. In some instances (e.g. Experiment IV) the unfertilized rate is greater during the last period than at the start. In other cases (e.g. Experiment II) it is fairly constant and in others (e.g. Experiment IX) it drops somewhat. But since the direction of change and relative magnitude is the same at both temperatures, the Q_{10}'s calculated in the first manner are not very greatly affected. Thus for Experiment IV we have 2.30, 2.22 and 2.35. The coefficients calculated in the second manner also are not greatly affected where the rate changes roughly uniformly. Thus in Experiment IV we have the Q_{10} values 2.36, 2.40, 2.19 and 2.24 for the individual vessels. It would, of course, be better to consider only cases in which there is very little change in rate. Taking Experiments II, V and VII as such, we get average Q_{10}'s of 2.70 and 2.77 which are closer to the values for fertilized eggs. We could not, however, find any criterion, such as the extent of agreement between duplicate vessels, the behaviour of the eggs upon fertilization, etc. that would justify the exclusion of any of the experiments listed.

The agreement between the coefficients calculated in the two ways described simply means that, where the rate of respiration changes, the change is fairly uniform. It does not, of course, measure the accuracy of the values.

The experiments at 20° and 10°, 18° and 8°, and 15° and 5° are listed in Table II. At these temperatures, the unfertilized eggs give

TABLE II

Eggs of Urechis. Same description as Table I.

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	° *C.*			° *C.*				
XII	1	20	1.74	1.84	10	0.73	0.68	2.55	
	1	10	0.93	0.88	20	1.99	2.10	2.26	
	1	20	2.20	2.17	10	1.00	1.02	2.16	
XIII	1	20	1.95	1.87	10	0.81	0.84	2.30	
	1	10	0.77	0.74	20	2.03	2.07	2.71	
	1	20	1.89	1.87	10	0.78	0.79	2.40	
XIV (no Kjeldahl)	1	20	32.3	(52.3)	10	12.3	(18.2)	2.63	2.87
	1	10	14.9	(20.4)	20	37.0	(52.0)	2.48	2.55
	1	20	31.8	(54.6)	10	11.9	(16.4)	2.67	3.33
Average								2.46	2.92
Average for individual vessels								2.47	2.82
Average for cleavage data									3.30
XV	2	18	2.01	1.97	8	1.10	1.10	1.81	
	2	18	2.28	2.25	8	1.05	0.90	2.32	
	2	18	2.92	2.80	8	0.83	1.00	3.13	
XVI	1	18	1.42	1.42	8	0.60	0.67	2.24	
	1	8	0.83	0.79	18	1.52	1.48	1.85	
	1	18	1.34	1.28	8	0.73	0.78	1.73	
Average								2.18	
Average for individual vessels								1.94	
XVII (no Kjeldahl)	2	15	46.4	(37.9)	5	16.9	(10.9)	2.75	3.48
	2	5	19.5	(11.2)	15	49.7	(36.0)	2.55	3.23
	2	15	55.5	(37.9)	5	15.5	(11.5)	3.58	3.29
Average								2.96	3.33
Average for individual vessels								2.84	3.30

consistently lower Q_{10}'s than do the fertilized eggs. In only two of the experiments were fertilized eggs run. For 20° and 10° no respiration experiments were available from previous work, but the coefficient for cleavage (3.30) which should be the same as for respiration (*loc. cit.*) is listed. For 18° and 8° and for 15° and 5° the cleavage value is

TABLE III

Eggs of Strongylocentrotus. Same description as Table I.

Vessels			A	B		C	D		
Experiment Number	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} unfert.	Q_{10} fert.
	hours	°*C.*			°*C.*				
I	2	22	3.39	3.60	12	1.30	1.35	2.63	
	2	12	1.23	1.48	22	3.23	3.24	2.39	
	2	22	3.75	4.20	12	1.46	1.58	2.61	
II	2	22	4.74	(16.4)	12	1.65	(6.8)	2.87	2.42
	2	12	2.03	(7.2)	22	5.23	(21.4)	2.57	2.97
	2	22	5.46	(22.1)	12	2.44	(10.6)	2.24	2.08
III (no Kjeldahl)	1	22	30.0	(64.7)	12	11.81	(29.5)	2.54	2.19
	1	12	13.6	(31.7)	22	32.29	(66.0)	2.37	2.08
	1	22	34.2	(66.4)	12	13.18	(30.1)	2.60	2.21
Average.								2.54	2.33
Average for individual vessels.								2.53	2.39
IV	2	20	2.61	2.65	10	0.67	0.70	3.84	
	2	10	1.22	1.22	20	2.52	2.95	2.24	
	2	20	2.96	3.23	10	1.33	1.34	2.32	
V (no Kjeldahl)	1	20	22.3	(22.7)	10	8.9	(10.9)	2.49	2.08
	1	10	11.0	(10.9)	20	23.8	(23.2)	2.46	2.14
	1	20	25.4	(23.2)	10	12.8	(13.8)	1.99	1.69
VI (no Kjeldahl)	1	20	24.4	(62.9)	10		(24.3)		2.59
	1	10	10.6	(29.0)	20		(64.4)		2.22
	1	20	26.7	(77.3)	10		(27.5)		2.81
VII	1	20	1.48	1.38	10	0.49	0.51	2.86	
	1	20	1.60	1.53	10	0.48	0.51	3.16	
	1	20	1.60	1.62	10	0.54	0.51	3.07	
VIII	1	20	1.03	1.00	10	0.33	0.30	3.22	
	1	10	0.40	0.42	20	1.20	0.92	2.59	
	1	20	1.09	0.96	10	0.55	0.39	2.18	
IX	1	20	1.24	1.27	10	0.42	0.52	2.67	
	2	10	1.19	1.27	20	2.89	2.88	2.35	
	1	20	1.72	1.82	10	0.65	0.67	2.67	
X	1	20	1.21	1.15	10	0.59	0.33	2.56	
	1	20	1.34	1.28	10	0.78	0.41	2.20	
	1	20	1.41	1.34	10	0.95	0.39	2.05	
Average.								2.63 ± 0.06	2.26
Average for individual vessels.								2.57 ± 0.05	2.23
Average from previously published data.									2.30

not given because *Urechis* eggs do not divide at 5° and only occasionally do so at 8°. By themselves, the experiments at these temperatures cannot be taken to demonstrate a difference between the coefficients for the unfertilized and fertilized eggs. They are, however, consistent with the results on the other forms investigated in giving at the lower temperatures somewhat lower values for the unfertilized eggs.

TABLE IV

Eggs of Strongylocentrotus. Same description as Table I.

Vessels			*A*	*B*		*C*	*D*		
Experiment Number	Time	Temperature	XO_2	XO_2	Temperature	XO_2	XO_2	Q_{10} unfert.	Q_{10} fert.
	hours	° *C.*			° *C.*				
XI (no Kjeldahl)	1	18	38.5	(48.2)	8	16.0	(16.7)	2.41	2.88
	1	8	15.5	(19.5)	18	38.5	(48.6)	2.48	2.50
	1	18	40.7	(55.3)	8	16.5	(19.1)	2.46	2.89
XII (no Kjeldahl)	1	18	24.4	(61.3)	8	9.3	(23.9)	2.61	2.56
	1	8	10.0	(27.8)	18	27.1	(65.6)	2.71	2.37
	1	18	27.7	(73.8)	8	10.0	(25.1)	2.77	2.94
Average								2.58	2.69
Average for individual vessels								2.59	2.57
XIII (no Kjeldahl)	1	17.5	19.8	(49.9)	7.5	7.4	(18.6)	2.69	2.68
	2	7.5	18.3	(38.4)	17.5	47.7	(106.3)	2.60	2.77
	2	17.5	54.6	(122.1)	7.5	20.1	(42.0)	2.72	2.91
Average								2.67	2.79
Average for individual vessels								2.69	2.80
								Q_{15}	Q_{15}
XIV (no Kjeldahl)	2	20	37.5	(121.6)	5	10.8	(28.9)	3.47	4.21
	2	5	12.0	(31.3)	20	44.6	(129.0)	3.72	4.12
	½	20	14.8	(39.8)	5	4.1	(9.2)	3.64	4.32
Average								3.61	4.22
Average for individual vessels								3.74	4.24

It is of some interest to compare the absolute values for the rate of oxygen consumption in different experiments. Considering only the first period in each experiment, we see in Table I that the rate varies from as low as 1.38 cu. mm. O_2 per hour per mg. N, as in Experiment VI, to as high as 5.06 cu. mm. as in Experiment XI. The differences are roughly correlated with the length of time that the animals were kept in the aquaria before the eggs were used, eggs from freshly collected animals giving higher absolute rates of respiration. Animals from different localities vary, but considering, for example, Experiments VI to XI in which the animals were collected at one time in one locality, the times after collection for numbers XI, VIII, X, IX, VII and VI are ½, 3, 4, 9, 10 and 40 days respectively, and the absolute rates of respiration decrease in just about that order. Eggs from the same animal were used in Experiments VII and XI. We shall not attempt an explanation at this time, but we may point out that *Urechis* does not store its eggs in the ovary (if there is a definitive ovary) but in "nephridial" sacs. Also, it is evident that simply aging the eggs in sea water produces the reverse effect, namely a rise in the absolute rate.

With the fertilized eggs we find no marked differences in the absolute rate of respiration. In addition to the figures of Table I we have ten more values for the absolute rate of oxygen consumption during the first hour after fertilization, all of which lie between 4.2 and 4.5 cu. mm. We find thus in *Urechis* cases in which the rate of respiration rises two or three-fold after fertilization, cases of no change in rate and cases (e.g. Experiment XI) of a decrease in rate. Whitaker (1933) showed that in different species the rate of respiration may rise, fall or remain unchanged after fertilization, the absolute rates for the fertilized eggs tending toward the same level in all. Here we have one animal exhibiting all three types of behavior.

Strongylocentrotus purpuratus

Tables III and IV give the respiration data and temperature coefficients for fourteen experiments with eggs of *Strongylocentrotus*. There are three at 22° and 12°, seven at 20° and 10°, two at 18° and 8°, one at 17.5° and 7.5°, and one at 20° and 5°. In all of them the rate of oxygen consumption of the unfertilized eggs shows a rise during the experiment. The shorter runs (e.g. Experiment III of Table III) show a smaller rise than do the longer runs (e.g. Experiment II).

In all of the experiments the temperature coefficients for the unfertilized eggs are fairly close to those for the fertilized eggs. For the temperatures 22° and 12°, the difference is small. For the temperatures 20° and 10°, it is somewhat greater. But if we omit the high values 3.84 and 3.22 of Experiments IV and VIII, the average Q_{10}, by the first method, is 2.27, which is much closer to the value for the fertilized eggs. An examination of the corresponding respiration figures shows that there is some justification for omitting these values, since, in both these experiments, vessels *C* and *D* give oxygen consumption figures that are evidently too low during the first period.

Table V

Eggs of Ciona. Same description as Table I.

Vessels			A	B		C	D		
Experiment No.	Time	Temperature	XO_2	XO_2	Temperature	XO_2	XO_2	Q_{10} unfert.	Q_{10} fert.
	hours	° *C.*			° *C.*				
I	2	25	16.02	14.84	15	9.32		1.66	
	1	15	4.64	4.04	25	9.77		2.25	
	1	25	8.71	8.28	15	4.76		1.79	
I	2	25	(29.72)	(29.36)	15	(13.57)	(14.01)		2.14
	1	15	(7.37)	(7.63)	25	(14.03)	(14.24)		1.88
	1	25	(19.01)	(19.09)	15	(7.31)	(7.07)		2.65
Average								1.90	2.22
Average for individual vessels								1.92	2.14
Average from previously published data									2.27
	hours								
II	1½	22	11.72	11.61	12	7.35	6.04	1.74	
	1	12	3.25	4.07	22	7.97	7.22	2.08	
	1½	22	9.78	11.28	12	4.19	5.47	2.18	
III	2	22	19.88	21.93	12	10.72	11.03	1.92	
	2	12	8.39	8.34	22	16.88	15.28	1.92	
	2	22	17.64	18.28	12	7.04	7.54	2.46	
IV	1	22	7.43	6.30	12	4.06	3.40	1.84	
	1½	12	3.16	4.55	22	8.89	8.28	2.23	
	1	22	6.29	5.06	12	4.68	4.86	1.19	
V	2	22	13.03	10.75	12	5.85		2.03	
	2	12	7.61	5.92	22	12.85		1.90	
	2	22	13.48	12.30	12	5.86		2.20	
V	2	22	(22.50)	(22.39)	12	(9.64)	(9.89)		2.25
	2	12	(9.93)	(9.56)	22	(20.94)	(20.41)		2.12
	2	22	(25.00)	(25.47)	12	(8.84)	(8.47)		2.92
VI	1	22	7.26	6.42	12	3.62	3.85	1.83	
	1	12	3.77	3.49	22	6.55	6.57	1.81	
	1	22	6.22	6.72	12	3.17	3.15	2.05	
VI	1	22	(9.55)	(9.30)	12	(4.16)	(3.67)		2.41
	1	12	(3.58)	(3.49)	22	(9.82)	(8.97)		2.66
	1	22	(10.90)	(10.04)	12	(4.08)	(3.85)		2.64
Average								1.96 ± 0.05	2.50
Average for individual vessels								1.98 ± 0.06	2.47
Average from previously published data									2.84

For the temperatures 18° and 8° and 17.5° and 7.5° there are also no significant differences between the coefficients for the unfertilized and the fertilized eggs, as the figures in Table IV show. One experiment at 20° and 5° shows some difference which may be significant. The unfertilized eggs give lower coefficients (Q_{15} in this case) than do the fertilized, which is consistent with the results on *Urechis* at the lower temperatures. The coefficients for cleavage which could be obtained more accurately than those for respiratory rate, are not given here because the eggs do not divide at 5° C.

Table VI

Eggs of Dendraster. Same description as Table I.

Vessels			*A*	*B*		*C*	*D*		
Experiment No.	Time	Temperature	X_{O_2}	X_{O_2}	Temperature	X_{O_2}	X_{O_2}	Q_{10} (unfert.)	Q_{10} (fert.)
	hours	° *C.*			° *C.*				
I	2	22	6.04	5.98	12	2.19	2.30	2.68	
	2	12	3.46	3.28	22	7.73	8.00	2.34	
	2	22	11.08	11.02	12	3.96	4.08	2.75	
II	2	22	4.40	4.05	12	1.96	2.08	2.09	
	2	12	2.72	2.31	22	6.10	6.19	2.44	
	2	22	10.80	9.51	12	3.39	4.17	2.69	
III	2	22	7.46	7.35	12	3.11	3.07	2.40	
	2	22	10.59	10.13	12	3.55	3.98	2.75	
	2	22	12.56	11.82	12	4.26	4.66	2.74	
IV	2	22	7.83		12	3.01		2.60	
	2	22	12.58		12	4.29		2.93	
Average								2.57 ± 0.04	
Average for individual vessels								2.71	
Average from previously published data									2.80

In the experiments of Tables III and IV marked "no Kjeldahl" the quantities of eggs employed were not determined, so the figures in these experiments cannot be compared with the absolute rates in the others. Also we cannot compare unfertilized and fertilized rates in those experiments, since the suspensions of unfertilized and of fertilized eggs were not of the same concentration. In the other experiments, if we compare the absolute rates of respiration for the unfertilized eggs during the first period of a run, we see no such differences as were obtained with *Urechis*. Here, the eggs used came from freshly collected animals. Whether keeping *Strongylocentrotus* in an aquarium tank would affect the rate of respiration of the unfertilized eggs was not determined.

Ciona intestinalis

Certain difficulties in handling *Ciona* eggs were previously (1936) pointed out. In addition it may be noted that to secure large quantities of unfertilized eggs it is necessary to use many individuals and to wash the eggs thoroughly before mixing them, in order to remove the sperm that almost unavoidably comes out with the eggs. Since *Ciona* eggs tend to float rather than sink to the bottom of the dish, it generally takes about ten or more washings to remove the sperm.

Six experiments were run with eggs of *Ciona,* one at 25° and 15° and five at 22° and 12°. The results are given in Table V. At the temperatures 25° and 15° there are no significant differences between the Q_{10}'s for the fertilized and the unfertilized eggs. In this experiment the vessels *A, B* and *C* were removed at the end of the unfertilized run, the eggs inseminated, and the measurements continued on the fertilized eggs. In addition a fourth vessel, *D,* of freshly inseminated eggs was added. Fertilization was 100 per cent in all. At the temperatures 22° and 12° the average Q_{10}'s are somewhat less for the unfertilized than for the fertilized eggs. Considering the magnitude of the difference in relation to the probable error and the fact that it agrees with *Urechis* and *Strongylocentrotus* in giving at the lower temperatures[3] lower coefficients for the unfertilized eggs, we are inclined to regard it as significant.

Comparison of the oxygen consumption figures for the unfertilized and fertilized eggs shows at most a less than two-fold rise upon fertilization. This is much lower than in the case of the sea-urchin egg. The rise is more of the order of that found with *Nereis* eggs (Whitaker, 1931; Barron, 1932).

Dendraster excentricus

Four sets of experiments were done on *Dendraster* eggs, all of them at 22° and 12° C. The results are presented in Table VI. In none were large enough quantities of eggs obtained to get usable oxygen consumption values in less than two hours at each temperature. *Dendraster* shows a fairly rapid rise with time in the rate of respiration of the unfertilized egg. The rate rises even more rapidly than in the case of *Strongylocentrotus* as Fig. 1 illustrates. In the last two hours of a six-hour run we may have more than twice the oxygen consumption obtained in the first two hours, as in Experiment II. The temperature coefficients, however, are not particularly affected by the rise. This means simply that the relative rise is about the same at different temperatures.

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[3] *Ciona* eggs are adapted to a higher temperature range than the others; they fail to divide below 12° and above 26° C.

The average Q_{10}'s for the unfertilized eggs by the two methods of calculation are 2.57 and 2.71 respectively. These are slightly lower than the previously determined value of 2.80 for fertilized eggs. The difference, however, cannot be taken as significant. The two average values for the unfertilized eggs differ more here than in the previous cases. That is because in two of the experiments (III and IV) coefficients could not be determined by the second method (from individual vessels) since the vessels remained at one temperature throughout the run.

TABLE VII

Average temperature coefficients (Q_{10}) *for unfertilized and fertilized eggs.* Probable errors given where sample consists of 15 or more values. Coefficients under *A* are determined from the respiration of eggs in different vessels run simultaneously at different temperatures. Coefficients under *B* are determined from the respiration of eggs in one vessel run consecutively at different temperatures. Coefficients under *C* taken from previously published data.

	Temperatures	Unfertilized eggs			Fertilized eggs			
		No. of vessels	*A*	*B*	No. of vessels	*A*	*B*	*C*
Strongylocentrotus	22° and 12°	8	2.54	2.53	4	2.33	2.39	
	20° and 10°	23	2.63 ± 0.06	2.57 ± 0.05	4	2.26	2.23	2.30
	18° and 8°	4	2.58	2.59	4	2.69	2.57	
	17.5° and 7.5°	2	2.67	2.69	2	2.79	2.80	
	20° and 5°	2	3.61†	3.74†	2	4.22†	4.24†	
Urechis	22° and 12°	34	2.57 ± 0.03	2.57 ± 0.04	6	2.64	2.65	2.79
	22° and 12°	12*	2.70	2.77				
	20° and 10°	10	2.46	2.47	2	2.92	2.82	3.30
	18° and 8°	8	2.18	1.94				
	15° and 5°	2	2.96	2.84	2	3.33	3.30	
Ciona	25° and 15°	3	1.90	1.92	4	2.22	2.14	2.27
	22° and 12°	19	1.96 ± 0.05	1.98 ± 0.06	8	2.50	2.47	2.84
Dendraster	22° and 12°	14	2.58 ± 0.04	2.71				2.80

* Selected experiments, included in line above.

† Q_{15} values.

DISCUSSION

The average values of the temperature coefficients for the eggs of the four animals investigated are listed in Table VII. As was pointed out in considering the individual cases, there are no large differences between the unfertilized and fertilized eggs. At the higher temperatures in each case, there are certainly no significant differences. At the lower temperatures, the consistently lower values for the unfertilized eggs of the different animals incline us to regard the difference as significant. With fertilized eggs, or for that matter most biological material (see Belehradek's review), Q_{10} increases as the temperature interval is lowered. Here, it appears that for the respiration of the unfertilized eggs, Q_{10} remains a constant or increases only slightly at lower temperatures. Thus, with *Strongylocentrotus* we get the values 2.54 at 22° and 12° and 2.68 at 17.5° and 7.5°. With *Urechis* the Q_{10}'s are 2.57 at 22° and 12° and 2.90 at 15° and 5°. We are not, however, particularly concerned here with the constancy of Q_{10}. Any other convenient measure of variation in rate with temperature would serve for comparing unfertilized and fertilized egg respiration.

We had expected to find the same values for unfertilized as for fertilized eggs. At the higher temperatures that appears to be the case. But if we accept the divergence at the lower temperatures as significant, then it would seem that one of the assumptions, upon which this expectation was based, must be wrong. This might well be the assumption that an unfertilized egg is a resting cell exhibiting only maintenance. Other processes besides what we term maintenance may be involved. It would seem important then to determine with certainty whether real differences exist at the lower temperatures. We do not, however, consider it likely that with the present material and methods simply expanding the experiments will improve the data very much. Besides, there now appear to be other ways of getting at the questions involved.

The unfertilized eggs of all the forms investigated exhibit a rising rate of respiration. In *Strongylocentrotus* and *Dendraster* the rate rises much more rapidly than in *Urechis* and *Ciona* (see Fig. 1), the difference being correlated with the time of loss of fertilizability on the part of the eggs. Runnström (1928, footnote p. 4) has likewise noted that the sea-urchin eggs lose their capacity for fertilization after exhibiting a spontaneous rise in respiration. There are several agents that have been reported to prolong the fertilizable life of the egg; namely, cyanide (Loeb, 1912) thyroxin (Carter, 1931), alcohol and dextrose (Whitaker, 1937). It would be of interest to know whether these agents would prevent the rise in respiratory rate of the aging unfertilized egg.[4] Under such conditions it is quite possible that different values for the temperature coefficients would be obtained.

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[4] Cyanide and CO suppress this rise according to Runnstrom (1930), but the concomitant fertilization test is not given.

Rubenstein and Gerard (1934) reported in *Arbacia* average values of 4.1 and 1.8 for the Q_{10}'s of unfertilized and fertilized eggs respectively. They point out, then, that as the temperature is increased the rise in respiration that occurs upon fertilization in the sea-urchin egg diminishes. By extrapolation they show that at 32° C. there would be no rise. In the sea-urchin, *Strongylocentrotus,* that we have used, as well as in the three other forms, no such differences in the coefficients are evident. At the lower temperatures, there are possibly significant differences, but in the reverse direction from what the above investigators find. However, if it is assumed that Q_{10} for the fertilized eggs decreases as the temperature rises, while Q_{10} for the unfertilized eggs remains constant, the unfertilized eggs would presently give the higher values. By extrapolation, then, if there is any point to it, we would find that the temperature, at which there would be no rise in respiration upon fertilization in *Strongylocentrotus,* approaches that of boiling sea water.

SUMMARY

1. The effect of temperature on the rate of oxygen consumption of unfertilized and fertilized eggs of *Urechis, Strongylocentrotus, Ciona* and *Dendraster* was investigated.

2. The unfertilized eggs exhibit a rising rate of respiration with time in all four species. The rise is much more rapid in *Strongylocentrotus* and in *Dendraster* than in *Urechis* and in *Ciona.* This rise appears to be correlated with the loss of fertilizability on the part of the eggs.

3. Methods of determining the temperature coefficients in such a way as to take into account the general rise (which is a significant factor in prolonged runs) and other variations are described.

4. Only the temperature coefficients for the same temperature intervals are compared, the respiration being determined at two temperatures in each experiment. With *Urechis* and with *Strongylocentrotus* eggs, experiments were run at temperatures between 22° and 5°; with *Ciona,* between 25° and 12°; and with *Dendraster,* 22° and 12°. The experiments with *Urechis* and *Strongylocentrotus* thus included low temperatures at which the fertilized eggs fail to cleave.

5. No significant differences between the temperature coefficients of the respiratory rate of unfertilized and of fertilized eggs of the four animals investigated are found over most of the temperature range in which development is possible. At the lower temperatures, there are differences that are possibly significant, the unfertilized eggs giving consistently lower values.

6. Comparison of the absolute rates of respiration of the unfertilized and fertilized eggs shows in *Strongylocentrotus* and *Dendraster* the rise

in respiration upon fertilization typical of the echinoids; in *Ciona* a less than two-fold rise is manifest; in *Urechis,* the rate may rise considerably, remain constant or decrease slightly, depending upon the particular batch of eggs employed. Eggs from animals kept some time in captivity give lower unfertilized rates and manifest a rise upon fertilization; eggs from freshly collected animals give higher unfertilized rates and no rise or even a slight decrease upon fertilization.

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SEXUAL AND ASEXUAL REPRODUCTION IN EUPLANARIA TIGRINA (GIRARD)

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Several authors have reported on the reproduction of *Euplanaria tigrina* (Synon. *Planaria maculata* Leidy), one of the commonest fresh-water planarians of North America. It has been known for a long time that this species often reproduces by fission. Curtis (1902), in his valuable paper on the life cycle of this form, also states that, at least in several localities, the animals grow sexually mature and deposit egg capsules in the early summer. Much material on the processes of fission has been gathered by experimental workers, particularly by Child and his co-workers. From these we know that the rate of fission may be controlled by external factors, such as temperature, the amount of food, and chemical properties of the water.

Nevertheless, the relations between the two manners of reproduction, the sexual and the asexual one, are as yet little known. The only extensive observations with regard to this question were undertaken by Curtis on material from the vicinity of Woods Hole, Massachusetts. This author investigated the life history of the species in four different localities through the course of three years and summarized his results as follows: "In some localities the species seems to have reproduced exclusively, so far as the observations go, by fission, in others only by the sexual process, while there are still others where both processes occur at different seasons" (1902, p. 556). In a later communication (Curtis and Schulze, 1924, p. 105) he writes: "It may also be noted that the differing habits of reproduction, originally reported . . . for the *P. maculata* in four different localities near Woods Hole, Massachusetts, have been confirmed by all our subsequent collecting in these localities."

These differences in the manner of reproduction in certain places suggested two possible interpretations: (1) there might be some differences in the physical and/or chemical characteristics of these localities, which influence the life cycles of the animals living there; or (2) there might be more than one physiological race of *Euplanaria tigrina*, showing different habits of reproduction. If the latter be the case, the manner of reproduction would be determined, chiefly, by internal factors.

It is the purpose of this paper to report on a series of experiments performed to analyze several factors that might control these processes. Most of the work has been carried out at the Miller School of Biology, University of Virginia. I wish to express my indebtedness to the Rockefeller Foundation which made this investigation possible, as well as my sincere gratitude to Professor William A. Kepner and Professor Ivey F. Lewis for the privileges extended to me during my stay at the University of Virginia.

MATERIAL AND METHODS

The animals used in the experiments were collected in four localities: (1) Sinclair's Pond, Park Street, Charlottesville, Virginia; (2) Big Spring, near Kerr's Creek, Rockbridge County, Virginia; (3) Mary's Lake, Naushon Island, Massachusetts; and (4) Pond south of Main Street, behind the Episcopal Church, Falmouth, Massachusetts.

In the experiments on asexual reproduction, the influence of temperature, hydrogen-ion concentration, amount of food, and irradiation with ultra-violet rays upon the rate of fission were investigated. The cultures were run in tap water.

Temperature experiments were performed in three series of cultures: at indoor temperature, low and high temperatures. The low temperature cultures were kept in electric, thermostatically controlled refrigerators, while high temperatures were obtained in simple, electrically heated ovens.

The experiments on the influence of the hydrogen-ion concentration in the culture water were carried out at indoor temperature. In order to keep the pH constant, small quantities (1 : 20) of buffer mixtures were added to the water. The buffers used were mixtures of KH_2PO_4 and NaOH having a pH of 6.4, 7.0, and 7.6, prepared after Clark's formulæ. Their addition to the tap water kept the media at a fairly constant acidity of respectively 6.5, 7.0, and 7.5.

The animals were fed with beef liver at regular intervals; it has been proved by various workers that cultures of fresh-water planarians may be run for years on this food. The liver was freely taken.

Ultra-violet irradiation was carried out by means of a Hanovia mercury-arc lamp kindly placed at the writer's disposal by the Committee on Effects of Radiation, National Research Council. These experiments did not yield any results bearing upon the process of reproduction; a more detailed description of the technique employed may, therefore, be omitted.

Experiments on Asexual Reproduction

For these experiments animals from Sinclair's Pond and from Big Spring were used. Each experimental series comprised at least three cultures containing, at the beginning, 5 to 10 specimens each. When fissions occurred, the posterior pieces were eliminated from the cultures. It is well known that the two pieces derived from fission, the head and the tail piece, behave differently in the regeneration of the missing parts. The head piece does not undergo any considerable change, its prepharyngeal part remains almost the same size, while the posterior end appears to grow out of it; the head piece, therefore, regenerates chiefly by epimorphosis. The tail piece, however, shows decided morphallaxis, i.e. it rearranges its proportions and a large part of the old tissues is directly transformed into parts of the missing prepharyngeal region (*cf.* Curtis, 1902, p. 529). Moreover, in a short time, before the regeneration is completed, the tail piece may redivide spontaneously. In head pieces, the intervals between two fissions are more regular. We may, with the necessary caution, take these intervals as indicative of the rate of reproduction. We must, however, realize that an absolute regularity in fission cannot be assumed. Although we select specimens of the same origin, the same age, the same size, and keep them in the same aquarium, yet a great variety of fission intervals will be observed. This is obvious in view of the fact that we cannot control all the factors concerned in the induction of fission. Among these, the nutrition of the individual specimens and their locomotory activity play important rôles. To eliminate the fluctuations of the fission intervals as much as possible, a sufficient number of specimens should be used for each experimental series and the cultures maintained for a sufficiently long time.

In order to express the frequency of fission in a convenient way, two data were calculated for each experimental series: the average interval between two consecutive fissions, and the average fission rate. The latter is the number of fissions per day, calculated for a lot of 100 specimens. It is a disagreeable fact that, in cultures running for a long period of time, the numbers of specimens can hardly be maintained constant, despite careful handling of the animals. The animals are apt to crawl up the wet walls of the culture dishes and then dry up if not noticed in time. Occasionally, individual specimens are lost while one is feeding and changing water. Therefore, the fission intervals and the fission rates were determined for shorter periods (usually 10 days) and from these data the average intervals and rates calculated for the entire length of observation.

Temperature Experiments

The indoor temperature varied considerably during the course of the experiments. In general, it fluctuated between 20 and 30° C., occasionally, for a short time, dropping below these limits (minimum 13.8° C., March 7, 1932) or rising above them (maximum 32.6° C., August 8, 1932). The results obtained in parallel cultures at indoor temperature and in the refrigerator are shown in Table I. At low temperatures (10–12° C.) the processes of fission were greatly inhibited or even entirely suppressed for several months. In a culture of 20 specimens (the number decreased, during the experiment, to 18) running 330 days, only five fissions occurred, the first of them on the 267th day. This shows that at least 13 specimens for almost 11 months did not display fission at all. The animals were well fed, so the decrease of the fission rate could not be due to starvation. On the contrary, in the culture referred to, the length of the animals was from 5 to 6 mm. at the beginning of the experiment, while at the end lengths of 16 to 20 mm. were measured. The control animals, raised at indoor temperature, generally remained smaller, since they divided before attaining the size of the refrigerator specimens. Another factor that might be suspected of preventing fission is the decreased locomotory activity in the refrigerator culture. The stimulating effect of light was eliminated in both series of cultures by keeping the aquaria in the dark. There is, however, a decrease of the activity caused directly by the low temperature. Nevertheless, since the animals in the refrigerator were often seen to move about in the aquaria, this difference

TABLE I

Low-temperature experiments. Average fission rate and fission interval in cultures kept in the refrigerator (10–12° C.) compared with those of control cultures at indoor temperature.

Origin of material	Number of specimens		Temperature	Duration of experiment	Average fission rate	Average fission interval
	Initial	Final				
				days		*days*
Sinclair's Pond	20	18	Refrigerator	330	.084	1189
Sinclair's Pond	15	4	Indoor	341	5.9	16.9
Big Spring	18	18	Refrigerator	329	.15	658
Big Spring	18	2	Indoor	329	7.2	13.9
Big Spring	15	14	Refrigerator	174	.041	2436
Big Spring	15	11	Indoor	174	6.0	16.7

can hardly be responsible for the extreme rarity of divisions at low temperature.

Several cultures were run at high temperature which varied between 29 and 34.5° C., in the main amounting to about 32° C. They showed a significant change in fission frequency during the course of the experiment (Table II). At the beginning, the fission rates were similar to those of the control cultures at indoor temperature. Soon the divisions became rarer and finally, after 43 days, no fissions occurred any more. Parallel with this decline in reproduction was a reduction of the size of the animals. The lengths of the specimens decreased from 7–13 mm. at the beginning to 3–5 mm. when the experiment was discontinued (after 55 days).

TABLE II

High-temperature experiments. Fission rate in cultures kept in the thermostat (about 32° C.), compared with those in control cultures at indoor temperature.

Origin of material	Number of specimens		Temperature	Fission rate		
	Initial	Final		1st–18th day	19th–36th day	37th–55th day
Big Spring.....	14	8	Thermost.	4.8	2.8	.81
Big Spring.....	12	11	Indoor	4.2	2.3	4.3

Unfortunately, I had no opportunity to determine exactly the optimum temperature for asexual reproduction. Nevertheless, observations on cultures at indoor temperature indicated that fissions were most frequent when the water temperature was about 25° C. or a little over.

Hydrogen-ion concentration

The observation of fissions in media of different acidity comprised only a small range of pH, viz. 6.5, 7.0, and 7.5. The addition of buffer mixtures to the culture water had no injurious effects on the animals. The reproduction continued in a normal way. No significant differences in the fission rates were observed in cultures of different controlled acidity, nor between them and the cultures in tap water (Table III). It may be noted, however, that the range of pH used was relatively narrow. It is known that planarians tolerate a considerable variation of acidity, towards the acid as well as towards the alkaline side.

TABLE III

pH experiments. Average fission rate and fission interval in buffered culture media and in tap water.

Origin of material	Number of specimens		pH	Duration of experiment	Average fission rate	Average fission interval
	Initial	Final				
				days		*days*
Big Spring.............	15	11	6.5	168	8.2	12.3
Big Spring.............	15	13	7.0	168	6.3	15.9
Big Spring.............	15	10	7.5	174	7.4	13.5
Big Spring.............	15	11	tap w.	174	6.0	16.7

Irradiation with Ultra-Violet Light

A number of specimens were exposed to the light of a quartz-mercury lamp on several consecutive days. During irradiation, the animals were kept in shallow dishes with little water. The animals so treated showed a slight decrease of the fission rate (Table IV). On the other hand, the irradiation apparently exerted a general injurious effect on the animals, which resulted in a high mortality in these cultures. I am, therefore, inclined to attribute the decrease of the fission frequency to the weakening of the animals rather than to a specific effect of the ultra-violet irradiation.

TABLE IV

Fission rates and fission intervals in cultures exposed to different doses of ultra-violet radiation and in control cultures not irradiated.

Origin of material	Number of specimens		Dose	Duration of experiment	Average fission rate	Average fission interval
	Initial	Final				
				days		*days*
Big Spring.....	15	7	Single	174	4.4	22.8
Big Spring.....	15	2	Double	174	4.6	21.7
Big Spring.....	15	11	—	174	6.0	16.7

Starvation

It is well known that planarians can stand long periods of starvation very well. In prolonged starvation they grow smaller and, to a certain extent, simplify their anatomical structure. Their physiological condition becomes that of young animals. It has been proved repeatedly that starvation causes asexual (as well as sexual) reproduction to cease. This is shown very clearly in Table V. For our experiments, well-fed specimens of a length of 12 to 15 mm. were taken. During the first week of starvation, fissions took place at an almost normal rate; later on, the frequency of division decreased rapidly. The last fission occurred on the twenty-second day after the starting of the experiment. On the sixty-fifth day the culture was discontinued. The size of the animals had decreased to 3½–6 mm.

I wish to point out that no traces of sexual reproduction or maturity were ever observed in any of the cultures of *Euplanaria tigrina* from Sinclair's Pond and Big Spring, either in the stock aquaria or among the specimens subjected to various external conditions.

TABLE V

Fission rates in cultures starving and in control cultures well fed.

Origin of material	Number of specimens		Nutrition	Fission rate			
	Initial	Final		1st–10th day	10th–20th day	20th–30th day	30th–65th day
Big Spring	30	21	Starving	9.7*	2.4	.3	.0
Big Spring	30	26	Fed	12.0*	4.0	4.3	5.1

* The high fission rates during the first days were due to the fact that fully grown specimens had been selected for the experiment.

EXPERIMENTS ON SEXUAL REPRODUCTION

Since sexuality could not be induced in material from two localities in Virginia, animals were procured from those places where Curtis (1902) had made his observations on the reproduction of the species. In July, 1932, asexual animals were collected in Mary's Lake; at the same time specimens brought in from the Pond in Falmouth proved to be sexually mature and laying egg capsules. Animals from these localities were kept in separate culture dishes, but under external conditions as identical as possible. They were raised at indoor temperature and fed on beef liver.

As a result, the two lots retained, in the main, their characteristic manners of reproduction also in the laboratory. The animals from the asexual locality continued to undergo fission. There were no decided seasonal cycles of reproduction observed, apparently because of the relatively favorable temperature all the year round. No sex organs developed in the course of 5 years.[1]

The animals from the sexual locality continued to propagate sexually in more or less regular periodical cycles. Though a definite coincidence of the periods of reproductive activity in the laboratory cultures with those observed in the field need not be expected, nevertheless the periodicity conformed, in the main, with Curtis' records of the life history in the natural habitat.

The duration of the egg-laying periods in indoor cultures varied within wide limits. Usually the animals started depositing the first egg capsules in the late fall or in winter (middle of November to February). The breeding reached its height in March and April, then declined, and generally ceased in June. Only once did I observe, in one culture, a breeding season extending from July till the beginning of September. Each specimen deposited several cocoons during one season. The size of the body gradually decreased from about 18–24 mm. to 13–15 mm. After the breeding season was over, the animals were rather sensitive, as if exhausted. The rate of mortality seemed to be higher at that time in the cultures. Nevertheless, most of the animals survived and recovered completely, if well attended to, and in the next egg-laying season again proceeded to produce cocoons. The individual lifetime of this species may be estimated at several years. Curtis' data on the degeneration of the sex organs between two seasons of sexual activity were confirmed.

Temperature Experiments

Like the fission of the asexual form, the rate of breeding in the sexual form is influenced by the temperature of the medium. This is clearly shown in Table VI. At low temperature the laying of cocoons proceeded very slowly; it was almost inhibited; at high temperature the breeding rate increased. On the other hand, the breeding season lasted much longer at low temperatures than at high ones. In the refrigerator culture referred to in Table VI, maintained at 10° C., single egg capsules were deposited at long intervals and the animals remained in the sexual state for at least one year.

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[1] One single seeming exception was noticed when, in March, 1933, one sexual specimen appeared in the asexual culture and laid two cocoons. This specimen was subsequently eliminated from the aquarium. Since then and up to now (May, 1937) no second case of sexuality has occurred. I am inclined to assume that this one and only mature animal had, by careless handling, been transferred to this vessel from the sexual culture while the dishes were being cleaned. Even though this assumption were not correct, the occurrence of one sexual individual among many hundreds of asexual animals would not be significant, and could not essentially affect the result of the experiment.

Besides reproduction by cocoons, the animals from the sexual locality also showed asexual reproduction by fission. Fission did not occur in all animals of the cultures and was confined to a short season of the year, from June to August. As this season followed the season of egg-laying, fissions were usually observed in animals which had previously deposited egg capsules. Young animals, hatched from cocoons in the preceding spring, likewise occasionally divided asexually, provided they had already reached a sufficient size and had not yet developed sex organs.

From the fact that the fissioning season coincides with the warmest season of the year, we may conclude that fission requires a high temperature. Temperature, however, is not the sole decisive factor: the animals must, at the same time, be in the state of sexual inactivity. If they are subjected to high temperature during the breeding season, they continue to deposit cocoons and do not divide.

TABLE VI

Temperature experiments on material from the pond at Falmouth. Daily breeding rate (calculated for 100 specimens) and breeding interval in cultures kept at indoor temperature, compared with those kept at lower and higher temperatures.

Temperature	Number of specimens	Duration of observation	Number of cocoons	Average breeding rate	Average breeding interval
		days			*days*
Indoor (about 20° C.)	20	31	29	4.68	21.4
Refrigerator (about 10° C.)	19	147	10	.36	279.3
Thermostat (about 28.6° C.)	9	35	98	31.1	3.21

Two tail-pieces of specimens which had fissioned after they had stopped laying egg capsules were studied anatomically. In both of them remainders of the copulatory organs were found, but in a state of apparent degeneration. The genital pore, parts of the atrial cavity, and the penis could still be identified, though their structure differed from that of the organs in function. Bursa and bursa stalk had entirely disappeared. In various places in the parenchyma of the genital region there were patches of brown substance, apparently the product of disintegration of tissues. In short, the pieces exhibited that typical picture of degeneration of sex organs which had been described by Curtis (1902, pp. 546–550) as occurring after the egg-laying.

I should like to emphasize again that fission, in the laboratory cultures, did not occur in the case of every individual specimen. The animals that did undergo fission usually divided only once. After the tail-piece had grown to a certain size, it occasionally divided a second time if the conditions were still favorable. In any case, fission plays only an insignificant rôle in the life history of the planarian from the pond at Falmouth.

The tendency to divide can be easily controlled by external factors, particularly those that influence the fission rate in the asexual form from Big Spring and Sinclair's Pond, i.e., temperature and nutrition. The following experimental series may illustrate this statement:

April 27, 1936, three cultures of 20 specimens each were prepared. The animals measured 18 to 24 mm. in length and were at the height of the breeding period.

Culture 1. Kept at indoor temperature, fed twice a week with beef liver. Egg-laying ceased June 12, fissioning started June 19 and lasted until August 3. Thirteen animals had divided, 7 remained undivided. These, measured September 21, were from 13 to 15 mm. in length. They entered a new breeding season on March 8, 1937.

Culture 2. Kept at indoor temperature, not fed. The last egg capsule was deposited on May 11; fissions occurred between June 30 and July 22. Only 3 specimens had fissioned, 15 were undivided, 2 had died. September 21, the animals measured 5 to 9 mm. From that time on they were regularly fed. The new breeding period started February 18.

Culture 3. Kept in a refrigerator at 10° C. Cocoons were laid at long intervals,—only 10 cocoons up to September 21, when the animals measured 15 to 21 mm. The egg-laying continued.

It is interesting to note that sexually active animals which had been kept at low temperature for a long time showed an extraordinary increase of the breeding rate when transferred to indoor temperature. This appeared in the continuation of the refrigerator experiment referred to in Table VI. Ten specimens which had been exposed to a temperature of 10° C. for 147 days had shown an average breeding rate of .36 (breeding interval of 279.3 days). Transferred to indoor temperature, they laid during the course of two months no less than 65 cocoons. This corresponds to a daily rate of 10.5 cocoons per hundred specimens or to a breeding interval of 9.5 days. Animals constantly kept at indoor temperature propagate at a much slower rate (see Table VI). From this experiment it would appear that low temperatures, while retarding the deposition of egg capsules at the time, do not inhibit the maturing of the germ cells to the same extent. Brought into normal temperature conditions the animals react to the accumulation of egg cells (and yolk material) in the body by an increased breeding activity.

Discussion and Conclusions

From the foregoing paragraphs it is evident that there must be at least two physiologically different races of *Euplanaria tigrina.* They differ, mainly, in their respective habits of reproduction. We may call them, shortly, the "sexual" and the "asexual" race. The different life cycles of this species in different localities near Woods Hole, Massachusetts, reported first by Curtis (1902 and 1924), are due chiefly to differences inherent in the animals themselves, not to physicochemical properties of the environment. The asexual race lives in Sinclair's Pond, Big Spring, and Mary's Lake; the sexual one in the pond at Falmouth (see p. 281).

Asexual reproduction has been observed in a relatively large number of fresh-water triclads. An excellent review of the material gathered has been given by Vandel (1921, pp. 370–374), who also assumed the existence of different races of *Euplanaria tigrina,* in which the manners of reproduction were hereditarily different. Few observations, however, have so far been made on the entire life cycles of fissioning planarians and little has been done to investigate the processes of reproduction in an experimental way. Nevertheless, it is a striking fact that in almost all species subjected to closer examination, differences in the incidence of sexual and asexual reproduction have been revealed in different localities (*cf.* Benazzi, 1936, p. 364). We know such physiological races in all European species of fissioning planarians: *Polycelis felina* (Dalyell) (Vandel, 1921, pp. 478–479; Thienemann, 1926, pp. 298–300), *Crenobia alpina* (Dana) (Vandel, 1921, pp. 478–479), *Euplanaria gonocephala* (Dugès) (Vandel, 1925; Benazzi, 1936), and *Fonticola vitta* (Dugès) (Beauchamp, 1932, pp. 285–294). Among the American species, sexual and asexual forms are known in *Euplanaria dorotocephala* (Woodworth) (Kenk, 1935*b*, p. 451). The same phenomenon is now confirmed in *Euplanaria tigrina.*

There is no reason to distinguish these races as separate taxonomic units, e.g. species or subspecies. Small morphological differences between them may occur, particularly in the shade of coloration, the size, and the proportions of the body; they are, however, of little taxonomic value. Some of these characteristics, such as the size and shape of the animals, may be directly correlated with the mode of reproduction.

The reproduction of the asexual form of *Euplanaria tigrina* is exclusively asexual. In this the field investigations of Curtis and the experiments described in this paper agree. External factors may accelerate the processes of propagation, retard, or even inhibit them, but they cannot change the process to a sexual one. The main factors influencing the rate of fission are temperature and nutrition. At low temperatures division is suppressed, it takes place only at about 10° C. or more, provided the animals have attained a certain minimum size. The interval between two fissions is shorter, the higher the temperature. During this interval, the anterior piece at least has to regenerate the posterior portion and therefore the nutrition of the animal plays an important rôle. The optimum temperature, though not exactly established, appears to be between 25 and 28° C. Above this temperature, the fission rate decreases. This decline probably is not the result of a reduced tendency to fission, but a sign of defective nutrition: at high temperatures the metabolism of the body is increased and the food taken does not suffice to cover the energy needs of both maintenance of body size and regenerative growth necessary to prepare the animal for the next fission. The animal is therefore in a state of inanition. This is seen also in the rapid decrease of the size. The maximum temperature tolerated continuously is a little above 30° C. (Lillie and Knowlton, 1898, determined this temperature for "*Planaria torva*" to be 32° C.; the species used in their experiments was probably identical with *Euplanaria tigrina*.)

There appears to be a correlation between the temperature and the size of the body at which fission takes place: at low temperatures the animals do not divide until reaching a considerable length, while at higher temperatures fission occurs already in smaller animals. This seems to apply to all fissioning planarians so far investigated. This phenomenon has not yet been subjected to a comparative study on a larger scale. Castle (1928, p. 420) e.g., observed it in another American planarian, *Fonticola velata*.

In their natural habitats, the life history of the asexual race of *Euplanaria tigrina* is simple: no reproduction in winter, fission during the warmer seasons. This cycle has been observed by Curtis (1902, p. 517) in "locality 4." Moreover, Taliaferro's (1920, p. 63) planarian from an abandoned ice-pond near the University of Virginia, in which reproductive organs were not observed for nine years, apparently belongs to the same race. This applies also to Hyman's (1920, p. 405) material from "the lagoon in Jackson Park in the city of Chicago" (p. 404).

The sexual race of *Euplanaria tigrina*, on the other hand, is capable of both sexual and asexual reproduction. Generally speaking, it develops reproductive organs and deposits egg capsules. Even if conditions be favorable throughout the year, sexual activity is not continuous, but occurs in certain physiological cycles. These cycles, in the field as well as in the laboratory (at indoor temperature), correspond to the seasons of the year. Apparently the yearly fluctuations of temperature are the decisive factor. The egg-laying season lasts several weeks or months. It goes on until the animals, especially their sex organs, are exhausted. Then follows a period of sexual inactivity during which the complicated copulatory apparatus first degenerates, to be newly reconstructed before the next breeding season. A detailed account of these processes has been given by Curtis (1902).

During the period of sexual inactivity the animals may undergo fission. This occurs only during the warm season of the year and is not at all a regular phenomenon. Many specimens remain undivided.

The regular life cycle can be influenced, particularly by the temperature. Low temperature retards the laying of cocoons, high temperature accelerates it. The formation of egg capsules appears to demand a certain minimum temperature, which lies not far below 10° C.

These data derived from laboratory cultures agree, in the main, with Curtis' observations in the field. Curtis (1902, p. 517) reports that *Euplanaria tigrina* in Locality 1 near Woods Hole, Massachusetts, lays egg capsules during May and June. After that, reproduction ceases and the reproductive organs degenerate. In August and September, i.e. in the season when the water is warmest, the animals are found reproducing by fission. Then reproduction is again suspended and a regeneration of the reproductive organs takes place during the winter.

In Locality 2, according to this author, the life cycle is similar to that in Locality 1, except that no fissions were observed there. There are several possible explanations of this fact: (1) the temperature may not rise so high as in Locality 1, i.e. it may not reach the minimum temperature necessary for fission in the asexual state; (2) the population in this place may belong to a physiologically different race with a fission temperature higher than that of the race from Locality 1 or even with the tendency to fission suppressed; and (3) there may be less food available in Locality 2, wherefore the animals may not reach the necessary size for fission. It would be desirable to investigate this question on the spot.

It is interesting to compare the data concerning *Euplanaria tigrina* with those observed in regard to other fissioning planarians. In cases where both manners of reproduction, the sexual and asexual, alternate according to the seasons of the year, the asexual phase always coincides with the warmer season. The majority of the species concerned develop sex organs preferably in the winter and early spring: *Polycelis felina* (Dalyell) and *Crenobia alpina* (Dana) (according to various authors); *Euplanaria dorotocephala* (Woodworth) (*cf.* Hyman, 1925, p. 65); *E. gonocephala* f. *subtentaculata* (Draparnaud) (see Draparnaud, 1801, p. 101, and Vandel, 1925, p. 502); *Fonticola morgani* (Stevens and Boring) (see Kenk, 1935*a*, p. 102); *F. velata* (Stringer) (see Castle, 1928, p. 419). In the natural habitats this rule is often obscured by two facts: First, certain localities have an almost constant temperature all the year round, e.g. springs, deep lakes, and subterranean waters; in these habitats often no alternation of reproduction takes place, though the animals in other surroundings would be capable of both sexual and asexual propagation. Secondly, there often occur physiological races, in which the tendency to sexual or asexual reproduction differs to a considerable extent (*cf.* Vandel, 1921, p. 478) and either one of them may be entirely suppressed; these latter forms, of course, are not considered here, since they have no alternation of reproduction.

The sexual race of *Euplanaria tigrina* appears to be different from the other fissioning forms in so far as it becomes sexually active in the warm season of the year. This difference is, however, only apparent. Here, as well as in the others, fission takes place at a higher temperature (August, September) than sexual activity requires (May, June). Only, the minimum temperatures necessary for either kind of reproduction are comparatively high.

It is a matter of further investigation to decide whether this analysis of the processes of reproduction, carried out for two races of *Euplanaria tigrina*, is valid for other forms of this species as well. It may be assumed that additional material will furnish a still greater variety of physiological characteristics.

SUMMARY

1. *Euplanaria tigrina* occurs in at least two physiological races which differ in the manner of reproduction: a sexual and an asexual race.

2. The asexual race, according to observations covering several years, reproduces exclusively by fission. Temperature and nutrition control the rate of fission but do not induce sexuality.

3. The sexual race periodically develops reproductive organs and lays cocoons. After the breeding period has ceased, the sex organs degenerate and fission may occur at high temperature. The individual animal can outlive several periods of sexual activity.

4. In nature, all planarians that have alternating (sexual and asexual) reproduction, appear to propagate sexually during the colder season and asexually during the warmer season of the year.

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INVESTIGATIONS AS TO THE LOCALIZATION OF THE MICROMERE-, THE SKELETON-, AND THE ENTODERM-FORMING MATERIAL IN THE UNFERTILIZED EGG OF ARBACIA PUNCTULATA

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I. Introduction

The available information about the localization of micromere-, entoderm- and skeleton-forming material, and about the stability of the egg-axis in the uncleaved egg of the sea urchin is not consistent. Driesch (1896, 1898, 1899, 1900) studied the cleavage of fragments (obtained by shaking) of unfertilized and fertilized eggs of *Echinus*. He came to the conclusion that the formation of micromeres is due to the cytoplasm, not to the nucleus. By way of explanation he suggested " ein polar-bilaterales Gerichtetsein der Teilchen " of the whole egg, but he also spoke of a certain local specific structure of the cytoplasm. Morgan (1894) found in part a migration of pigment, in part a formation of micromeres at the vegetative pole of *Arbacia*, even when the furrows were formed in an atypical sequence. Boveri (1901*b*) called attention to the vegetative polar cap and its specific qualities, and Hörstadius (1928, p. 14) confirmed the results of Boveri: vegetative and meridional halves of *Paracentrotus* may show a typical cleavage, whereas the animal half does not form any micromeres. By removing vegetative fragments of different sizes from the unfertilized *Paracentrotus* egg, Hörstadius (*loc. cit.*, p. 18) proved that the potentiality of forming micromeres gradually decreases from the vegetative pole towards the animal and ceases about halfway between the normally micromere-forming area (unpigmented) and the equator. The micromere-forming region is thus restricted to the unpigmented pole-cap and the lower half of the pigment ring material in *Paracentrotus*. (Boveri (1901*b*, p. 155) also saw a pigmented micromere.) Furthermore, Hörstadius (*loc. cit.*, p. 15) found, by aid of the pigment band, that the cleavage axis in fragments remained unchanged, the micromeres both in meridional and vegetative fragments being formed from the unpigmented pole-cap.

Whereas Driesch (1900 etc.) in his early papers, spoke of all parts of the sea urchin or starfish egg as equipotent, Zoja (1895), Terni (1914), v. Ubisch (1925) and Hörstadius (1928) found that animal halves of cleavage stages could not gastrulate, nor form any skeleton. Boveri (1901*b*, p. 158, 1902) used the pigment ring of *Paracentrotus* to study whether the polarity remains unchanged in fragments of unfertilized eggs. Animal fragments (without pigment) did not gastrulate. One vegetative and one meridional fragment gave larvæ in which the pigmented and unpigmented regions were differentiated in such a way as to show that the polarity of the fragments was not altered. Hörstadius (1928, p. 33) confirmed these results. Animal halves of both fertilized and unfertilized eggs of *Paracentrotus* developed in the same ways as animal halves of 8- or 16-cell stages. They do not gastrulate, nor do they form skeleton. Meridional and vegetative fragments, on the other hand, invaginate archentera and produce spicules. Thus not only the micromere-forming, but also the archenteron- and skeleton-forming material is restricted, in the unfertilized *Paracentrotus* egg, to the vegetative half. Isolation of animal and vegetative fragments of the 64-cell stage in a plane corresponding to the middle of the pigment ring region (between veg_1 and veg_2, Hörstadius, 1935, p. 319) demonstrated that the upper level of the skeleton-forming area corresponds roughly to that of the micromere-forming material in the uncleaved egg. This upper limit of the skeleton material was not determined in detail in the unfertilized egg (*cf.* above as to the micromere-forming material). By aid of the pigment ring Hörstadius (1936*a*) also showed in agreement with Boveri, that the polarity of fragments of the unfertilized eggs does not change.

These investigations on *Paracentrotus* have proved that both the micromere-, entoderm-, and the skeleton-forming material is localized in the vegetative part, roughly speaking, the vegetative quarter, of the unfertilized egg, and that the polarity of the egg remains unchanged in fragments, both as regards the cleavage axis and the differentiation axis.

Some results obtained by other investigators on different material conflict considerably with this view. Harnly (1926) found the cleavage pattern of fragments of unfertilized eggs of *Arbacia* dependent, not upon the orientation of the plane of fragmentation in relation to the egg-axis, but in relation to the nucleus. Boveri (1901*a*) stated that the pronucleus of the mature egg of *Paracentrotus* may have any position in relation to the egg-axis. Harnly (1926) and Hoadley (1934) found the same thing in *Arbacia*. Thus neither the animal, nor the vegetative half is noticeably preferred. From the different cleavage patterns resulting on dividing eggs in different planes with relation to the nucleus, Harnly comes to the conclusion that the micromere-forming material prior to fertilization lies in the region between the pronucleus and the center of the egg. A nucleated fragment as large as half an egg, or larger, cleaved as does the whole egg. When an egg was divided into two equal halves in the plane shown in Fig. 2, the non-nucleated fragment did not form any micromeres. But when the egg was cut in a plane close to the nucleus, it was the non-nucleated fragment that cleaved as a whole egg. Shortly after fertilization Harnly found a different arrangement. Now only the half containing the egg nucleus will divide. Both this and the uncleaved half remained inside the intact fertilization membrane. Fragments that must have been meridional and vegetative cleaved as whole eggs, and other fragments segmented as animal halves. These results from fertilized eggs agree with those of Boveri and Hörstadius, although Harnly's interpretation is somewhat different (see p. 309),—Harnly studied only the cleavage pattern; he did not follow the further development.

Taylor and Tennent (1924), Taylor, Tennent and Whitaker (1926), and Tennent, Taylor and Whitaker (1929) obtained essentially different results for *Lytechinus* (*Toxopneustes*) *variegatus*. The polar bodies and the funnel (micropyle, made visible by octopus ink) served as landmarks for orientation. These authors state that a new polar axis is established in fragments. With a few exceptions, the first two planes of cleavage were at right-angles to the surface of section, regardless of the orientation of the cut, and the micromeres were formed on the cut side at the end of the intersections of these two planes. Nor is there a complete segregation of the presumptive primary mesenchyme or entoderm in the vegetative part of the mature, unfertilized egg. Their experiments indicated that, prior to fertilization, a mesenchyme-entoderm-forming substance has a uniform distribution through about 19/20 of the egg; the animal polar cap alone seemed to contain only ectoderm material. In the larger part of the egg, however, the presumptive entoderm and mesenchyme was supposed to be restricted to the interior, surrounded by a superficial layer of ectoderm-forming material, as small superficial fragments never gastrulated. Thus both animal, vegetative, meridional and oblique halves of *Lytechinus* could form not only micromeres, but also mesenchyme, and could develop to plutei. In most cases a new polar axis was established, perpendicular to the cut side.

In August, 1936, when spending the summer as a fellow of the Rockefeller Foundation at the Marine Biological Laboratory, Woods Hole, I had the opportunity of testing the localization of the micromere-, the entoderm-, and the skeleton-forming material in the egg of

Arbacia punctulata. Because of the short time available for this investigation the material examined was scanty, but as the results of the observations are very definite, I think they are worth publishing. They indicate that localization in the *Arbacia* egg does not differ from that in *Paracentrotus.*

The eggs were cut with a fine glass needle manipulated by hand. In order that the eggs should not slip away during the sectioning they were placed in a little scratch in a piece of celluloid (Hörstadius, 1928). The cut was orientated either in relation to the pronucleus (Fig. 2), or to the micropyle, which was made visible by ink from the squid (*Loligo*) (Fig. 1).

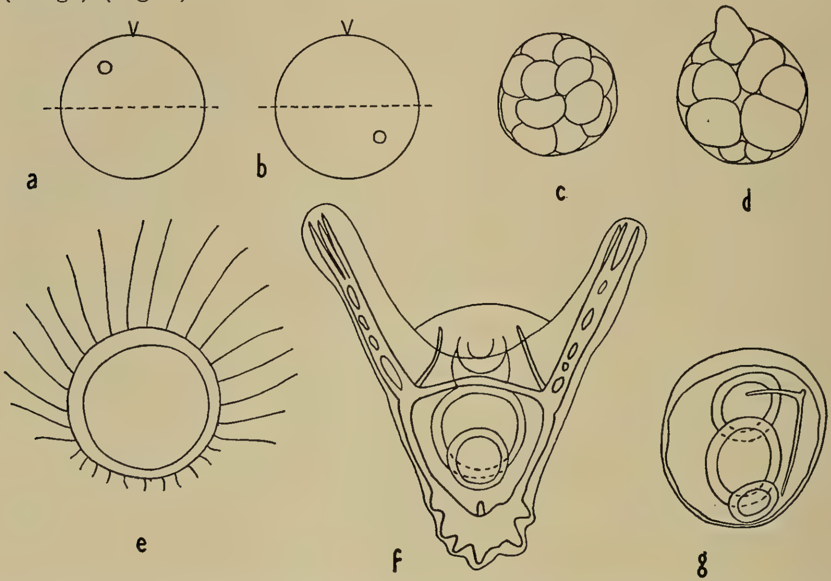

FIG. 1. Cleavage and differentiation of animal and vegetative halves of the unfertilized egg of *Arbacia punctulata.* Orientation by means of the micropyle (*a*, *b*). The egg nucleus may lie in the animal or the vegetative half (*a*, *b*). *c.* Equal cleavage of the animal half. *d.* The vegetative half has formed macro- and micromeres, the latter at the original vegetative pole, antipolar to the cut side, where a cytoplasmic lobe has protruded through a slit in the fertilization membrane. *e.* Differentiation of the animal halves into blastulæ with enlarged apical tuft. *f*, *g*. The vegetative halves gastrulate and form skeleton, developing to ovoid larvæ (*g*) or plutei (*f*). The results indicate a segregation of the micromere-, the entoderm-, and the skeleton-forming material in the vegetative part of the egg.

II. ISOLATED ANIMAL AND VEGETATIVE HALVES, AND MERIDIONAL HALVES

Twelves eggs were divided equatorially. At the operation the pronucleus in some cases lay close to the plane of section, in other cases far away from it. In 7 pairs the egg nucleus lay in the animal half, in 5 in the vegetative (Figs. 1*a*, *b*). The animal and the vegetative halves of each egg were reared separately and kept in pairs. Of the twelve pairs only one vegetative fragment died early.

A. Differentiation

Zoja (1895), Boveri (1901*b*), Terni (1914), and others have stated that animal fragments do not gastrulate and possess a very enlarged apical tuft. This phenomenon was studied in greater detail by Hörstadius (1935, pp. 283–314).

None of the twelve animal halves gastrulated. They all developed considerably enlarged apical tufts, thus differentiating completely in conformity with animal fragments in *Paracentrotus* (Fig. 1*e*). On the other hand, all the vegetative fragments gastrulated and formed skeletons. Some of them developed into good plutei with mouth and arms (Fig. 1*f*), the others showed a more vegetative type with ovoid body shape, no mouth, and a poor skeleton (Hörstadius, 1928, 1935) (Fig. 1*g*).

As controls for the animal and vegetative halves four pairs of meridional halves were also isolated. In two pairs only one partner developed. The other six larvæ (two pairs and two single larvæ) all gastrulated and formed skeletons, developing into more or less typical plutei, or only ovoid larvæ.

B. Cleavage

After fertilization of egg fragments a fertilization membrane forms in the usual way. In *Arbacia* the membrane is not raised from, but lies close to the surface of the egg. When, on cleavage, the volume of the egg increases because of the division into blastomeres, the membrane often bursts on the cut side, and a lobe of cytoplasm protrudes through the opening (Harnly, 1926; Tennent, Taylor and Whitaker, 1929, p. 18; Hörstadius, 1936*b*, p. 820). Sometimes this cytoplasmic bud is nearly cut off by the edges of the membrane, but very often it is connected with the egg by a rather broad base. Nuclei may migrate into the lobe, which may thus be divided into cells. In both cases the bud indicates the cut side.

Of the 12 vegetative halves 11 formed micromeres. Morgan (1894) found that the red pigment in the *Arbacia* egg in the 4-cell, seldom in the 2-cell stage, migrates away from the vegetative pole. As a consequence the micromeres are lighter and whiter than the other blastomeres. The micromeres in our vegetative halves were of this whitish type. But there were not always 4 micromeres (only in seven cases). In one case there was only 1, in two cases 2, and in one case 3 micromeres. One egg showed an irregular cleavage,—it was the vegetative half that died. It is a well-known fact that the formation of micromeres may be suppressed, even in an entire egg, without any influence on the later development. Thus the primary mesenchyme is not dependent on the presence of micromeres during the cleavage. The suppression of micromeres is often caused by a slight mechanical disturbance (Boveri, 1901*b*; Hörstadius, 1928, p. 124). The absence of one, two, or three micromeres is probably due to such secondary influences.

The essential point is the relation of the cytoplasmic bud to the micromeres, i.e. the position of the micromeres in relation to the egg-axis. In three eggs no lobe was formed, in all the other eight cases the cytoplasmic bud was antipolar to the micromeres (Fig. 1*d*). This shows, without any exception in the cases where the landmark was visible, that the micromeres were formed at the original vegetative pole.

The cleavage pattern of the meridional halves was not so regular. In one pair both halves had cells of somewhat varying sizes, but no real micromeres were observed. In another pair one fragment had all cells of equal size, those of the other being slightly irregular. In the remaining two pairs, one half had no small cells, the other two either light micromeres close to the bud or only one small cell, the character of which is uncertain. (In the absence of the cytoplasmic bud its position could not be determined.)

Eleven of the 12 animal halves showed cells of equal, or slightly varying sizes, but no micromeres (Fig. 1*c*). The twelfth fragment possessed two pairs of small cells, but they cannot be regarded as real micromeres, as they were not lying close together.

C. CONCLUSIONS

In dealing with the often irregular cleavage patterns of fragments, we have to define what is meant by a micromere. Not every small cell is a micromere. Not even every small, whitish cell. As we shall find below, small whitish cells can be formed, owing to particular factors, so that we cannot speak of real micromeres. We only define as micromeres those small, whitish cells which have been formed by a process of fundamentally the same character as in the normal egg.

The isolation of animal and vegetative halves of the unfertilized egg of *Arbacia* gave exactly the same results as with *Paracentrotus.* Animal halves show partial cleavage (no macro- and micromeres are formed, Fig. 1*c*), and differentiate as isolated animal halves typically do, with enlarged apical tuft, and without gastrulation (Fig. 1*e*). The vegetative halves segment as whole eggs, and without any rotation of the egg-axis (Fig. 1*d*). The micromeres are formed at the original vegetative pole, opposite the cut (equatorial) side, thus not on the cut side as in *Lytechinus*. The vegetative halves of *Arbacia* also differentiate as isolated vegetative halves of other species do, giving ovoid larvæ or plutei (Fig. 1*f*, *g*). This characteristic cleavage and differentiation of animal and vegetative halves takes place irrespective of whether the larvæ are haploid or diploid, and irrespective of the position of the pronucleus of the egg in relation to the plane of section. The results thus indicate a segregation of the micromere-, the entoderm- and the skeleton-forming material in the vegetative part of the egg.

The meridional halves also support this view. All surviving fragments gastrulated and formed spicules. It is important to note that micromeres were not formed very regularly. In the only positive case did they lie close to the cytoplasmic bud, thus close to the cut side, as one would expect. This absence of micromeres is probably due to the fact that the mechanism of micromere formation—especially in the unfertilized egg—is very sensitive to mechanical injury (Hörstadius, 1928, pp. 19, 124), and that the cut passes through the supposed micromere-forming region. In *Paracentrotus* I obtained micromeres also in meridional halves in most cases, but there the membrane is less tight, or perhaps the vegetative cytoplasm less sensitive. It is to be regretted, that more cases were not available for study.

III. Fragments of Equal Size Isolated by a Cut as Far Away from the Nucleus as Possible

To test Harnly's results, unfertilized eggs were divided into two approximately equal halves by a cut as far from the nucleus as possible. Eggs were chosen in which the nucleus lay fairly close to the periphery. The plane of section was perpendicular to a line through the nucleus and the center of the egg and passing through the latter (Fig. 2*a*). All the micromere-forming material which, according to Harnly, lies between the nucleus and the center of the egg, should be found in the fragment containing the egg nucleus. The plane of section thus is at random in relation to the egg-axis. Fifty-one pairs were reared.

A. Differentiation

Not all the fragments started to develop after fertilization; some remained undivided. A few happened to get lost. But in 35 pairs both partners reached full differentiation (Fig. 2). In 13 only one of the two fragments developed. In 18 pairs both fragments gastrulated and formed skeletons, developing into plutei or ovoid larvæ. In 5 the diploid partner differentiated as an animal half (enlarged apical tuft, no invagination), the haploid as a vegetative (archenteron, skeleton, ovoid larva or pluteus), and in 6 pairs I found the reverse, the haploid fragment behaving as an animal half. In addition there were 3 cases in which the diploid had only a small archenteron, with or without skeleton, but the haploid a typical or large archenteron, and skeleton, whereas in another 3 pairs the reverse was the case (the haploid larvæ with a small invagination—Fig. 2). The 13 single larvæ developed as follows: 7 diploid, 2 haploid larvæ with more or less typical archentera and skeletons; 1 diploid gastrula with too small invagination; 1 diploid and 2 haploid blastulæ with enlarged apical tuft.

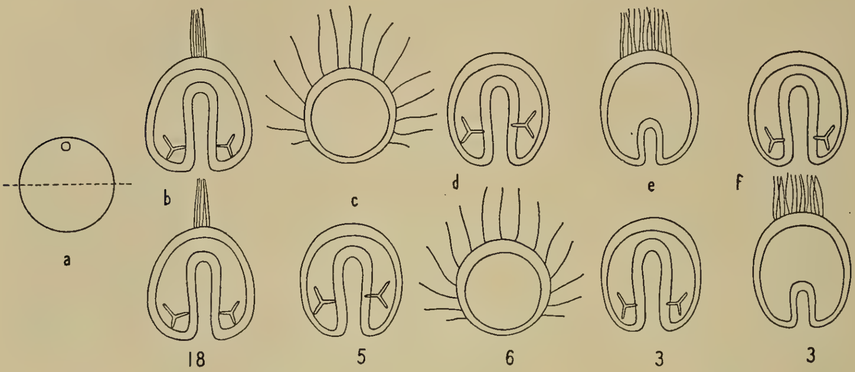

FIG. 2. Diagram of the differentiation of halves of the unfertilized egg of *Arbacia* isolated by a cut perpendicular to the line nucleus-center of the egg. The cut thus at random in relation to the egg-axis. In 18 pairs both partners gastrulated and formed skeleton, developing like approximately meridional halves (*b*). In 11 pairs the one larva differentiated as an animal half, which in 5 of those cases was diploid (*c*), in 6 haploid (*d*). In 3 pairs the diploid, in 3 the haploid fragment developed into a gastrula with too small archenteron (*e, f*). The results demonstrate a localization of entoderm- and skeleton-forming material in a part of the unfertilized egg that must be smaller than half the egg, and independent of the position of the egg nucleus.

B. Cleavage

We have already seen that the cleavages of the meridional halves were somewhat atypical, as the micromere formation was often inhibited. Moreover, the blastomeres may often vary more or less in size. In the halves isolated by a cut at random in relation to the egg-axis, we find all sorts of cleavage patterns.

It has been emphasized above that probably not all small cells observed are micromeres. On the contrary, small cells evidently may be formed as a result of factors fundamentally different from those leading to the formation of the typical micromeres. One has to be very careful regarding the interpretation of the nature of the small cells. Sometimes the cytoplasmic bud, protruding through the opening of the fertilization membrane—although not containing any nuclei or real cell membranes—may at the first glance look like a micromere, or a group of micromeres. In some cases the cell membranes at one or two divisions may also pass through the cytoplasmic lobe, but the small "cells" formed in that way will still lack nuclei. Very often, however, a nucleus migrates out into the bud and subsequently divides there. After two divisions, the lobe is divided into four small (if the lobe was small) cells, which are similar to micromeres, in that they are lighter than the other cells (the cytoplasmic bud is often not so markedly pigmented as the other part of the egg). When the lobe does not protrude much outside the membrane, these cells have a striking similarity to micromeres. But we can also find small cells, not micromeres, inside the membrane, but formed in connection with the bud. If the cytoplasmic bud is pressed out in the 2- or 4-cell stage, one of the quarter-blastomeres may be much smaller than the other ones. After two further divisions its descendants will constitute four small cells inside the fertilization membrane (or two or three, if one nucleus has migrated into the lobe, Fig. 3*a*). The bud may remain or fall off. This group of apparent micromeres on the cut side are thus formed by a process essentially different from that of the normal formation of micromeres. Occasionally the first cleavages in fragments may produce blastomeres of more or less unequal size with or without the formation of a bud. In that way, after several divisions, the smallest blastomere may give rise to a quartet of small cells, without any particular relation to the egg-axis, or to the cut side. The small cells of probably non-micromere origin may be of any number from one to five or six, or even more. It seems to me, moreover, that the pressure of the edges of the membrane on the base of large buds produces small cells, not only as descendants from a small half- or quarter-blastomere, but as a result of the pressure at the cleavage leading to

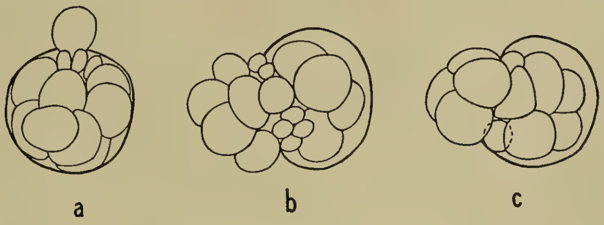

FIG. 3. *a*. Formation of small cells at the cut surface, because one of the earlier blastomeres has lost the greater part of its cytoplasm by a cytoplasmic protrusion. At the following divisions the descendants of the part left inside the membrane naturally were much smaller than the other blastomeres. *b*, *c*. Formation of small cells under the edge of the membrane, single (*c*) or in groups (*b*) at a distance from each other.

the 8- or 16-cell stage. This appears more probable in that several times small cells were found just under the edge of the membrane, and not always as a single group, but separated from each other (Fig. 3, *b*, *c*).

Thus there are several possibilities of obtaining small cells, often of a lighter color than the other blastomeres, and these small cells are not real micromeres. One would expect from this that small cells, micromeres and atypical small cells, would occur much more frequently in fragments than normally. But the contrary is the case. As mentioned above (pp. 300 and 301), micromere formation is very sensitive to mechanical injury; e.g. after shaking, an egg may show an equal cleavage, but the differentiation will not be influenced. In *Arbacia* fragments the micromere-formation seems to be inhibited very often.

As a result of these experiences, we have to deal with the following possibilities when dividing the unfertilized egg at random, if we assume the same arrangement of micromere material as in *Paracentrotus.* Vegetative and meridional halves ought to give whole cleavage, animal halves equal. But any fragment may cleave equally, i.e. if the micromere formation has become inhibited. Animal halves, and the other types of halves with inhibited micromere formation may show a more or less irregular pattern, the cells varying slightly in size. Any kind of fragment may form small cells which are not micromeres, either from the bud itself, close to the bud, or at any other place. It is naturally not easy to draw any conclusions from experiments where there are so many possibilities! According to Taylor, Tennent and Whitaker in *Lytechinus* all micromeres should appear on the cut side. According to Harnly, only the diploid halves form micromeres.

One diploid and 3 haploid fragments showed whole cleavage, with a bud opposite the micromeres. They thus behaved like the vegetative halves (p. 300 and Fig. 1*d*). Two diploid and 2 haploid fragments cleaved as whole eggs, having a lobe more or less on the side: they correspond to more or less meridional halves. Six diploid and 4 haploid fragments formed typical macro- and micromeres, as far as I could judge, but without any bud that made possible an orientation. Nine diploid and 5 haploid fragments cleaved equally, with or without bud. In 6 diploid and 4 haploid fragments the size of the blastomeres varied more or less, but no cells were as small as micromeres. In 7 diploid and 10 haploid fragments I found one or several small cells, the nature of which is open to question. They were in most cases formed either from or near the lobe, but sometimes not close to the lobe, or at a distance from each other.

These observations are at variance with those of Taylor, Tennent and Whitaker on *Lytechinus*, as micromeres were sometimes formed at a distance from or even opposite the cut side, and they are not in agreement with the results of Harnly on *Arbacia*, as of the cases regarded as typical cleavage 9 were diploid while the same number, 9, were haploid.

Let us now examine the relations between cleavage patterns and differentiation. We speak below of three kinds of larvæ (Fig. 2). One corresponds to the animal halves of *Paracentrotus* and of *Arbacia* as we have seen in the preceding chapter, a blastula with enlarged apical tuft. One constitutes a gastrula with a large archenteron and with skeleton, derived from vegetative, meridional, and oblique vegetative-meridional halves. It is not essential to mention whether the larva later becomes a typical pluteus or only an ovoid larva. The important point is whether it gastrulates or not. The third type is a gastrula with a too small archenteron, such as is derived from oblique animal-meridional halves (Fig. 2, *e*, *f*).

Of the 18 fragments above characterized as showing whole cleavage, 17 developed to gastrulæ with large archentera and skeletons; one gave a blastula with enlarged apical tuft. The 24 fragments with equal (14) or slightly unequal (10) cleavages differentiated into 6 blastulæ, 2 gastrulæ with small, and 16 with large archentera. This result is what would be expected, as I consider that these two cleavage types correspond to animal halves and other types of half with suppressed micromeres. The 17 dubious cases must be described more in detail. In one pair one fragment showed two, the other one small cell inside the membrane, near the bud, but not quite in contact with it. Both developed into gastrulæ. I am inclined to interpret those as real micromeres, the halves as meridional fragments. In another case, some small light cells were formed at some distance from the bud, about 45°. The fragment differentiated into a gastrula. The nature of the cells is unknown. In one case the lobe itself divided into two small cells and one large cell. The former are certainly not micromeres, as the latter lay between them. The development led to a blastula with enlarged apical tuft. Three fragments had three or four small cells lying inside the membrane, just under the bud (Fig. 3*a*). I assume that these cells were so small because the expulsion of the bud deprived one of the quarter-blastomeres of a large part of its cytoplasm. Another reason for believing that these small cells are not micromeres is that the other blastomeres cannot be classified as meso- and macromeres. All three fragments gastrulated, but one of them had a slightly enlarged apical tuft and only a small archenteron. In four cases a large part of the fragment had protruded through the opening in the fertilization membrane and divided, just as well as the part within the membrane. In all four fragments small cells were formed, and in all they were situated under the edge of the membrane (Fig. 3*b*, *c*). I do not believe that these small cells are comparable to micromeres, as in three of the four fragments they were found single or in groups at a distance from each other (Fig. 3*b*, *c*). One developed into a gastrula, two into blastulæ of the animal type, and one died. The remaining six fragments had one or several small cells, but the sizes of the other cells were so varied, and the position of the "micromeres" sometimes so scattered, that we cannot speak of a regular cleavage pattern with micromeres. In one case a close examination showed that the two apparent micromeres were not cells at all, only two small buds without nuclei. Three of these six fragments gastrulated, two developed into blastulæ with enlarged apical tufts, and one died.

C. Conclusions

In this experiment we divided the egg with a random section in relation to the egg-axis, the plane of section being oriented in relation to the egg-nucleus (Fig. 2). The entoderm- and the skeleton-forming material cannot be limited to the region between the egg nucleus and the center of the egg as both the diploid and the haploid larvæ form archentera and skeletons with about equal frequency (in 27 and 26 cases respectively, from the pairs) and as the blastulæ with enlarged apical tufts were derived not only from haploid (6 cases) but also from diploid fragments (5 cases). Nor can the micromere-forming material be localized between the egg-nucleus and the center of the egg, as Harnly stated. Of the 18 cleavage stages that were considered to possess typical micromeres, 9 were diploid and just as many—9—haploid. Fifteen diploid and 9 haploid fragments formed no micromeres, showing equal or slightly irregular cleavage. In 7 diploid and 10 haploid fragments, small cells were found, the nature of which has been discussed in detail.

As 5 diploid and 6 haploid fragments of the pairs and 1 diploid and 1 haploid of the single halves failed to gastrulate, the entoderm- and skeleton-forming material cannot have the same widespread distribution in *Arbacia* as in *Lytechinus*, in which, according to Tennent, Taylor and Whitaker, it occupies 19/20 of the volume of the egg. In that case every healthy fragment of the size of a half-egg would have gastrulated. Furthermore, the micromeres were not always formed on the cut side. In 4 cases they were found antipolar to the cytoplasmic bud, in 4 this bud was situated laterally in relation to the cleavage axis. In 24 fragments no micromeres were formed. In some of the 17 dubious cases we could show that the small cells on the cut side were not real micromeres.

On the contrary, our results with one exception are in conformity with the view that both the micromere-forming and the entoderm- and skeleton-forming material is localized in a part of the *Arbacia* egg that is smaller than half the volume and comparable to the vegetative quarter of the *Paracentrotus* egg. Both diploid and haploid halves could gastrulate or differentiate into blastulæ of the animal type. Both diploid and haploid halves could form micromeres. These could appear on the cut side, opposite the cut side, or on any other side. With one exception, all fragments that formed "real" micromeres gastrulated. Fragments with equal or slightly irregular cleavage gave all kinds of larvæ, in accordance with the view that they comprise the animal halves and any other kind of halves with suppressed micromeres. Of the dubious cases, some with small cells developed into blastulæ with enlarged apical tuft, i.e. as animal halves; but it was shown above that in all probability those small cells were not real micromeres, as either the whole cleavage stage was very irregular, or the small cells were located at some distance from each other. The only case that does not fit in with the assumption of a localization of both the micromere-forming and the entoderm- and skeleton-forming material in the vegetative part of the egg was one fragment which was supposed to have a typical 16-cell stage but differentiated into a blastula with large apical tuft (p. 305). Was that a case of micromere formation of an animal half, or was the interpretation of the cleavage stage wrong?

IV. Discussion

Although the micromere- and the skeleton-forming material occupy the same region in the *Paracentrotus* egg,—roughly speaking, the most vegetative quarter—the factors causing the formation of the micromeres and those causing the formation of the primary mesenchyme of the skeleton-forming cells are not identical. As many authors have pointed out, normal primary mesenchyme is formed even if the micromeres are suppressed.

Harnly (1926) studied only the localization of the micromere material, but we have also paid attention to the differentiation of the fragments which were isolated in order to test his results. The outcome of his experiments, that the micromere-forming material in *Arbacia* prior to fertilization lies between the nucleus and the center of the egg, is contradicted by the two series of experiments described above. Animal halves did not form any micromeres, whereas the vegetative fragments did, and in both cases irrespective of the position of the egg nucleus: the animal and the vegetative fragments could be diploid or haploid; the egg nucleus could at the operation lie close to the plane of section or at some distance from it. When the egg was divided into two equal halves by a section as far from the nucleus as possible, Harnly (see his Table 3 and Fig. 1*a*) obtained normal cleavage only in the nucleated fragments (23), whereas 28 haploid fragments showed "two tiers of eight equal cells." The pairs described above formed (in the fairly clear cases) micromeres in 9 diploid and 9 haploid, and no micromeres in 15 diploid and 9 haploid fragments.

In the light of my experiences, I have difficulty in understanding Harnly's results. He found in his experiment (Table 1) that "in no case did a nucleated half that divided normally through the first three cleavages give other than a normal fourth cleavage." Of 132 diploid fragments 30 showed normal fourth cleavage, 38 irregular and partial first cleavage, 50 endoplasmic buds (not studied), and 9 were undetermined. In Tables 3–5 no buds and no irregular and partial first cleavage are recorded. When the eggs were fragmented, as in our Fig. 2*a*, practically all diploid fragments segmented normally, but the haploid formed "two tiers of eight equal cells." When the eggs were divided into two equal halves by a section close to the nucleus, all the fragments that were determined, diploid or haploid, segmented in a normal way, except a few haploid ones, which had only two or three macro- and micromeres. As we have seen, I have not found that fundamental difference between the diploid and haploid fragments cut as in Fig. 2*a*. Both kinds could form micromeres, and both kinds could show equal or slightly irregular cleavage. When equal, the blastomeres were hardly ever arranged as regularly as in two tiers of eight cells, as Harnly states for his 28 enucleated halves. Harnly also isolated non-nucleated fragments containing two-thirds or more of the material of the egg. One fragment was undetermined, 18 segmented normally. Harnly's explanation is that these large haploid fragments contained all the micromere-forming material lying between the nucleus and the center of the egg. A more or less typical cleavage would in this case be expected also when assuming a localization of the micromere material in the vegetative quarter of the egg, since only very few of the fragments would be completely devoid of such material.

Harnly (1926) also divided fertilized eggs into two equal halves. According to Harnly, the fusion nucleus is to be found in the egg-axis, just above the equator; Harnly tried to cut equatorially. The fertilization membrane could not be divided, the two halves remained inside the membrane, flattened against each other. Only the half containing the egg nucleus segmented. Three types of cleavage were found: whole cleavage with the micromeres opposite the cut side, whole cleavage with the micromeres close to the cut side, and equal cleavage. Harnly concludes from his experiments that, within five minutes after fertilization, the micromere-forming material changes its position. Both the nucleus and the micromere material are now oriented with regard to the primary axis. Harnly does not expressly state where along the axis the micromere material is now located, but he seems to indicate a central position. As far as I can see, his results accord just as well with the view that the micromere material is restricted to the vegetative part of the egg. The equal cleavage (the largest number, as the nucleus in most cases was lying above the equator) belongs to the animal halves; the whole cleavage with the micromeres opposite the cut side corresponds to the cases in which the nucleus came to lie in the vegetative half; and the two fragments with the micromeres close to the plane of division are probably, as Harnly himself presumes, meridional halves.

Tennent, Taylor and Whitaker (1929, p. 66) state that their results "have compelled the conclusion that in the unfertilized egg of *Lytechinus* there is an animal polar cap and a superficial layer of ectoderm-forming material surrounding a core of undifferentiated material that is potential endoderm, primary and secondary mesenchyme, and mesoderm." The presence of an animal cap of ectoderm-forming material, occupying 1/20 of the volume of the egg (*loc. cit.*, p. 66) was concluded from the fact that gastrulæ were obtained from vegetative fragments of 1/11 the volume of the egg (but there is only one of that size, No. 1926 253), while none of the larvæ from fragments of equal size from the region of the animal pole developed beyond the stage of blastulæ with mesenchyme (No. 1926 276, 279; see also pp. 47, 63, 64, 66). The smallest fragment that developed into a blastula was 1/21 of the volume of the egg. These results point, as it seems to me, rather to the size 1/11 than 1/20 for the animal polar cap. The reason for the conclusion that there is a superficial layer of presumptive ectoderm also in the other parts of the egg is not given in the paper in question, as far as I can find. But in the preliminary report by Taylor, Tennent and Whitaker (1926), the same conclusion has been drawn from the fact that small superficial fragments—with a diameter of one-fifth of that of the egg—only gave blastulæ with or without mesenchyme, while larger, but still small fragments gastrulated. A fragment with a diameter of one-fifth of that of the egg has a volume that is only 1/125 of that of the egg. Nobody has so far been able to rear gastrulæ from such small fragments. The smallest blastomere that has been observed to gastrulate is a half-macromere (Hörstadius, 1936*a*). Its volume is larger than 1/32 but smaller than 1/16 of the egg. Morgan (1895) and Driesch (1900, 1902) estimated the smallest egg fragment that could gastrulate at 1/40–1/60 and 1/32 respectively of the volume of the egg. But these results are very uncertain, as the size of the fragment was concluded from the size of the larvæ. In the full report, Tennent, Taylor and Whitaker (1929, p. 62) state, contrary to the preliminary note, that the smallest fragment that gave a gastrula had 1/11 the volume of the egg, the smallest that gave a blastula 1/21 (*cf.* above). Under such circumstances I cannot see how the idea of a superficial layer of ectoderm-forming material round the whole egg can be supported. Very small fragments probably do not gastrulate because they are too small, not because of lack of presumptive archenteron (if derived from a vegetative part of the egg). As the smallest blastulæ obtained were derived from fragments measuring 1/21 of the volume of the egg, no blastulæ seem to exist that could prove the presence of a superficial layer of ectoderm-forming material outside the entoderm-mesenchyme-forming substance that, in *Lytechinus*, is supposed to occupy 19/20 (10/11?) of the egg.

We now turn to a comparison of the facts leading to the idea of an animal cap of ectoderm material, occupying only 1/20 (1/11?) of the egg in *Lytechinus*, with the facts demonstrating that in *Paracentrotus* and *Arbacia* the presumptive ectoderm is distributed over more than half the egg. Animal halves of the unfertilized egg of *Paracentrotus*, oriented at the operation by means of the pigment ring, never gastrulated—*cf.* p. 296. None of our 12 animal halves of *Arbacia* (orientation by means of the micropyle) gastrulated, and all showed an enlarged apical tuft. Of the halves cut at random, many differentiated in the same way (Fig. 2). Tennent, Taylor and Whitaker (1929, p. 57) report the history of 27 pairs of fragments isolated by horizontal sections. But of these pairs, both fragments reached gastrulation age only in 9 cases. If the presumptive entoderm- and mesenchyme-forming material occupies 19/20 (10/11?) of the egg, every horizontal fragment larger than 1/20 (1/11?) should gastrulate. This was the case in 6 pairs, the relative size of the fragments varying from 1 : 1 to 1 : 3.4 (No. 1924 8, 42, 44, 54, 61, 1926 230). In one case both gastrulated with the size difference 10 : 1 (No. 1926 253), the animal fragment being the larger. In two pairs, however, the animal partner did not gastrulate. In the one pair its volume was only 1/17 of that of the egg (No. 1926 229), but in the other the fragments were of approximately the same size (1 : 13, No. 1926 231). The animal fragment formed a blastula without mesenchyme. In addition, 3 of the single larvæ, all of half size (No. 1924, 5, 45, 79) remained as blastulæ, while 11 gastrulated. These results, with 4 fragments of approximately half size differentiating only to blastulæ, are at variance with the assumption that the presumptive entoderm and mesenchyme occupies 19/20 or even 10/11 of the egg.

Of 21 vertical sections in *Lytechinus* both partners developed to gastrulation age only in 5 pairs. Here we should expect gastrulation in every fragment of sufficient age (10 hours), and it occurred in 4 pairs (No. 1924 40, 53, 56, 80). But in the fifth pair (No. 1924 60) one fragment only formed a "blastula with mesenchyme," while the other developed to a pluteus. These two fragments were of equal size. Furthermore, not less than 3 (No. 1924 23, 25, 1926 267) of the other 10 vertical halves (only one partner living) that lived longer than 10 hours failed to gastrulate.

Seven pairs of fragments obtained by diagonal sections (Tennent, Taylor and Whitaker, 1929, p. 58) were reared (No. 1926 275, 279–283, 285). In four cases the small, oblique animal fragment, of size 1/6, 1/11, 1/11, 1/21, formed a blastula with mesenchyme, while the larger vegetative fragment gastrulated. In two cases the animal fragment was the larger (5 : 1, 4 : 1); both partners gastrulated. In the seventh pair also both developed to gastrulæ, the size of the oblique animal fragment being 1/7 of that of the vegetative.

The blastulæ obtained from the animal fragments are said to have a thickened posterior wall and often a number of mesenchyme cells. No mention is made of the size of the apical tuft in these blastulæ. Nevertheless, I get the impression from the drawings of such blastulæ (Figs. 44, 47) that they are typical animal fragments. Thus the thickened wall would correspond, not to the posterior, but to the animal and ventral side (*cf.* Lindahl, 1933; Hörstadius, 1935, p. 286, 1936*a*, p. 56). This interpretation is supported by the fact that the "mesenchyme" cells in the figures are located further towards the thin wall than towards the thickened wall. Furthermore, I very much doubt whether all the cells reported as mesenchyme cells have that character. In fragments, very often some cells pathologically migrate into the blastocœl. Many isolated animal halves of *Paracentrotus* may have a few, or a large number of cells free in the interior of the blastula. They have nothing to do with real mesenchyme cells. They may, of course, also appear in meridional and vegetative fragments, together with primary mesenchyme cells. It requires an intimate knowledge of the material to recognize the real mesenchyme cells from these pathological cells, and also to interpret correctly the animal blastulæ. The cells in the blastulæ (Figs. 44 and 47) are rather varied in size. This indicates, it seems to me, that here we have not to deal with real primary mesenchyme. Furthermore, the assumption that the mesenchyme material occupies 19/20 of the egg is contradicted by the fact that Tennent, Taylor and Whitaker report several blastulæ of half size without mesenchyme (No. 1924 5, 23, 25, 1926 231).

The fact that 4 of the horizontal and 4 of the vertical fragments of half-size did not gastrulate is incompatible with the conception of the presumptive entoderm occupying 19/20 (10/11?) of the egg. It rather shows that it is restricted to less than the half of the egg. Also the small diagonal fragments illustrate the same thing, as in some cases they contain material from the equator region without gastrulating.

The fact that both partners did gastrulate in 7 of the 9 animal-vegetative pairs, and that 4 of 20 vertical fragments did not gastrulate, seems to indicate that the orientation was not always that desired. When discussing the localization of the entoderm and mesenchyme material in *Arbacia* with some colleagues at Woods Hole in 1936, I said that one ought to obtain blastulæ of animal type from, roughly speaking, 15 per cent of the halves isolated at random, if the same conditions as in *Paracentrotus* prevailed. My material of random sections on *Arbacia* numbers 14 blastulæ with enlarged apical tuft and 65 larvæ that gastrulated. Thus 18 per cent did not gastrulate. This indicates that the presumptive entoderm occupies less than half of the egg, otherwise the animal blastulæ would have been much fewer. Eight of the *Lytechinus* fragments of approximately half-size remained in the blastula stage, while 40 developed into gastrulæ or plutei. Thus 17 per cent did not gastrulate. These figures of all the horizontal and vertical sections together raise some doubts as to the accuracy of the orientation of the cut, particularly as 4 of 20 meridional fragments only formed blastulæ. Has the plane of section often been different from that desired? One possibility is that the egg slipped or turned during the operation. The three authors state (1929, p. 6) that the operator could control this. The micropyle and the polar bodies served as landmarks. Are they reliable? Tennent, Taylor and Whitaker (1929, p. 12) regarded it as possible that the egg might rotate in its jelly and in that manner make the micropyle of uncertain value as a landmark. "No evidence of rotation was found. Careful examination showed the polar bodies lying at the base of the micropyle. In one instance only were they out of line as much as five degrees." But in the preliminary report (1926) it is stated that "our preliminary observations having convinced us that the egg might be rotated in its jelly, we felt that our orientation of the egg was dependable only when the polar bodies could be found at the base of the micropyle." But is it not possible that the egg can rotate inside the jelly, the polar bodies being held by the micropyle? (Hörstadius, 1928, p. 91.) If so, the egg-axis may lie at any angle to the axis indicated by the micropyle and the polar bodies. Or has the whole egg rotated during the slow sectioning with the micromanipulator?

From the fact that, in certain fragments obtained by vertical section, the first cleavage amphiaster lies temporarily in the short axis of the cell, and that in those cases the size of the cleavage cells is atypical, Tennent, Taylor and Whitaker (1929, pp. 41–48) conclude that there is a prelocalization of an entoderm-forming substance. I can but support the reservation of Dr. Taylor (*loc. cit.*, p. 47), who doubts the significance of cell-size as indicative of a segregation of entoderm-forming material in the unfertilized egg. In *Paracentrotus*, under certain circumstances, the four animal cells of the 8-cell stage may be much larger than the four vegetative (vorzeitige Mikromeren), or the spindles of the second and third division may stand obliquely, or the spindle of the first division in an animal-vegetative direction instead of equatorial, or a vegetative half may show a typical whole cleavage, all without any change of the position or amount of not only the micromere-, but also the entoderm-forming material (see Hörstadius, 1928, pp. 10, 14). As far as I can see, the results in question only permit conclusions as to the cleavage factors, not as to the localization of presumptive entoderm.

Both Harnly (1926) and Tennent, Taylor and Whitaker (1929) state that the first two furrows in fragments stand at right angles to the surface of section, irrespective of the orientation of the egg. The last-mentioned authors maintain that a new polar axis has been established (*loc. cit.*, p. 66). But, as we have seen above, the position of the first two furrows may be entirely independent of the polar axis of the egg and larva. In order to determine whether a new polar axis has been established, one has to ascertain by local vital staining whether the cleavage axis coincides with the axis apical organ—blastopore in the gastrula. In *Paracentrotus* the micromeres are always formed at the vegetative pole, irrespective of the position of the first two furrows, and differentiation takes place in accordance with the original egg-axis (Hörstadius, 1928, p. 10; 1936*a*, p. 53). Also, if micromeres are formed on the cut surface at the intersection of the first two cleavage planes, as stated for *Lytechinus*, this does not necessarily mean a change of polarity, as the factors for micromere formation and skeleton and entoderm formation are not identical (*cf.* the paragraph above and p. 307).

The statement that the micromeres are always formed on the cut side in *Lytechinus* is surprising. Tennent, Taylor and Whitaker have studied the cleavage of fragments in a great many cases, and the drawings seem convincing, although they look rather schematized. But the statements are perhaps not entirely conclusive, as the micromeres in our vegetative *Arbacia* fragments were formed opposite the cut side and as the three authors (1929, pp. 36–39) also made some experiments on *Arbacia* at Woods Hole, which do not agree with those presented here. Only 14 of 45 operations were either fully or partially successful. If we count all the fragments that developed, single and pairs, 1–4 micromeres were found in 6 vegetative and 6 animal fragments, while 2 vegetative and 1 animal fragment cleaved irregularly, and 5 animal and 2 vegetative divided equally. Nothing is said as to the position of the micromeres in relation to the cut surface. These results contrast markedly with mine: 11 of 12 vegetative fragments showed micromeres, all opposite the cut side, while the animal halves did not form any real micromeres. Tennent, Taylor and Whitaker do not discuss any of the possible sources of error regarding the micromere formation. Moreover, perhaps the free hand is a better instrument for cutting the eggs than the micromanipulator, which may work so slowly that a rotation of the egg after the final orientation is possible. A renewed investigation on *Lytechinus*, as well as on *Arbacia*, with attention payed particularly to the orientation of the cut and the possible formation of false micromeres, with a large number of larvæ that reach full differentiation, and with local vital staining to study the relation of the axis of the egg to that of the fragment and the relation of the micromeres to the cut surface, would be very desirable.

The critical discussion may be *summarized* as follows. Harnly's assumption (1926) that the micromere-forming material in the unfertilized egg of *Arbacia* is situated between the nucleus and the center of the egg does not hold, as vegetative halves formed micromeres, and animal halves showed no micromeres, in both cases irrespective of the position of the nucleus, and as nucleated and non-nucleated halves isolated by a cut perpendicular to the line nucleus-center of the egg (Fig. 2*a*) formed micromeres with equal frequency. The conclusions of Tennent, Taylor and Whitaker (1929) that the egg of *Lytechinus* has an animal polar cap occupying only 1/20 (1/11?) of the volume of the egg, and a superficial layer of ectoderm-forming material are contradicted by their own results. The conclusion is drawn from the differentiation of one animal and two vegetative fragments of the size 1/11. But there are diagonal fragments of the same size, or larger, reaching down to the equator, that do not gastrulate. Moreover, several animal and meridional fragments of half size and sufficient age did not gastrulate. These facts indicate that the presumptive ectoderm occupies at least half the egg. The fact that several meridional halves also differentiated as an animal fragment arouses the suspicion that the plane of section has not always been that desired, which would explain many of the discrepancies between *Lytechinus* and *Paracentrotus*. It is questionable whether some of the cells described as mesenchyme cells are not pathological. The position of the first two cleavage planes does not necessarily indicate the position of the axis of the larva. In fragments of *Lytechinus* eggs the micromeres are reported by Tennent, Taylor and Whitaker to be formed on the cut side. In *Arbacia* I found the micromeres formed at the vegetative pole, irrespective of the position of the section. The fact that the results of Tennent, Taylor and Whitaker as to the localization of the micromere-forming material in *Arbacia* do not agree with mine, which in turn confirm those on *Paracentrotus*, makes a reinvestigation of *Lytechinus* also desirable.

V. SUMMARY

Animal halves of unfertilized eggs of *Arbacia punctulata* segmented equally and developed into larvæ of animal type: blastulæ with enlarged apical tuft. Vegetative halves formed micromeres antipolar to the cut side, and gastrulated, differentiating into ovoid larvæ or plutei. Meridional halves all gastrulated. The type of development was independent of the presence or absence of the egg nucleus (Fig. 1).

Unfertilized eggs were divided into approximately equal halves by a section perpendicular to the line nucleus-center of the egg (Fig. 2*a*), the plane of section thus being laid as far away from the nucleus as possible, and at random in relation to the egg-axis. Nucleated and non-nucleated fragments formed micromeres and gastrulated with equal frequency. Some of the fragments differentiated as animal halves (Fig. 2). Atypical small cells, particularly on the cut side, which may be mistaken for micromeres are often formed.

The results indicate that the micromere-forming and the entoderm- and the skeleton-forming material in *Arbacia* is located in the most vegetative part of the unfertilized egg, occupying less than half the volume of the egg. The results of Harnly (1926) on *Arbacia* and of Tennent, Taylor and Whitaker (1929) on *Lytechinus* are critically discussed.

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EXPERIMENTS ON DETERMINATION IN THE EARLY DEVELOPMENT OF CEREBRATULUS LACTEUS

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I. INTRODUCTION

The eggs of nemerteans, annelids, gastropods, lamellibranchs and scaphopods show the spiral type of cleavage. Most of these eggs,—those of the annelids and mollusks,—are assigned to the mosaic type of determination. Their isolated blastomeres have been found to develop in accordance with their prospective significance. None of these eggs has, however, been investigated in such detail as the sea urchin egg, of which we know that some parts may develop into a complete larva (Driesch, 1891, etc.) and that other parts may differentiate less typically than in the case of normal development, owing to the fact that interactions have to take place between different parts of the egg, bringing the formation of some organs (Hörstadius, 1928, 1935, 1936). [von Ubisch (1936) has denied the rôle of interactions in the sea urchin egg. However, new isolation and implantation experiments, as well as constriction experiments (unpublished), completely confirm my earlier results as to the interactions.] The scope of this investigation was to see whether parts of an egg of the mosaic type might not show traces of interaction when transplanted atypically upon each other. As I could not find any annelid or mollusk egg so suitable for isolations and transplantations as the *Cerebratulus* egg, I had recourse to the latter. This egg shows a spiral cleavage but is not wholly a mosaic egg. It belongs to the regulation type insofar as fragments of the uncleaved egg and isolated half- and perhaps quarter-blastomeres may give rise to harmonic dwarf larvæ. But, on the other hand, the investigations of E. B. Wilson (1903), Zeleny (1904) and Yatsu (1910) indicate that the animal and vegetative parts of cleavage stages differentiate as fragments. Thus in that respect we seem to have to deal with a mosaic.

The cell-lineage of the nemertean egg has never been worked out. For that reason, in order to be able to interpret rightly the results of the isolation and transplantation experiments, we had first to investigate the prospective significance of the cell layers. As the experiments of the authors mentioned above are rather incomplete, we also have had to repeat and extend the study of isolated fragments. The results of previous investigators will be mentioned below in connection with each corresponding experiment.

II. Material and Methods

The investigation was undertaken in July and the first part of August, 1936, at Woods Hole. The worms (*Cerebratulus lacteus* Verrill) were sent in several deliveries in ice-cooled jars from Lynn, north of Boston, and kept in an ice-box. In order to obtain the eggs, a piece of a female was put into a piece of cheesecloth in sea water. The cheesecloth retains the slime but allows the eggs to fall through. Coe (1899) has shown that the germinal vesicle gradually fades away, when the eggs come out in the sea water and the first polar spindle is formed, but the spindle remains in the metaphase until fertilization occurs. In order that uniform conditions as regards maturation and fertilization might be secured, the sperms were added half an hour after the shedding of the eggs (Zeleny, 1904). The egg forms no fertilization membrane but is surrounded by a jelly and a soft membrane, which are easily removed by gentle shaking.

The blastomeres of the 2-, 4-, and 8-cell stages are easily separated with glass needles. The separation of the four layers of the 16-cell stage is more difficult, as the blastomeres from two adjacent layers lie partly between each other (Fig. 1, *B*). But it can be done by cutting before the fourth cell division is quite completed, because at that moment the cells have not yet sunk in between each other.

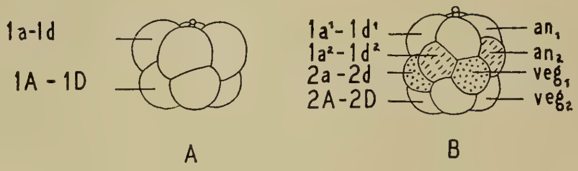

Fig. 1. Eight-cell stage (*A*) and 16-cell stage (*B*) of *Cerebratulus.*

For transplantation the fragments were placed one on top of the other in a cavity of appropriate size, made in a celluloid plate, and a small glass sphere placed on top of the upper fragment to produce pressure (Hörstadius, 1928). In some cases the fragments fused after being made to adhere by a brief, gentle pressure of the needle. Local staining (of a single blastomere or of a layer of blastomeres) was made by leaning the cell or cells in question against a piece of agar (Vogt, 1923, 1925; v. Ubisch, 1925) or by staining an isolated cell layer and then transplanting it (Hörstadius, 1928, 1935).

The number of experiments is rather limited. This is due partly to the fact that the termination of the breeding season already in early August cut short my experiments before the completion of an adequate study, partly to the fact that in many series nearly all larvæ died.

The reason for this was not a pathological condition of the larvæ. They looked very healthy until they suddenly disappeared by getting caught and expanded, just as if exploded, by the surface tension. The death ratio was considerably reduced when the larvæ were reared in a large drop of water between a slide and a coverslip on plasticine feet and protected from evaporation by vaseline.

III. Normal Development. Nomenclature

The following facts regarding the normal development have to be borne in mind. The *Cerebratulus* egg is very dark brown, opaque. But the polarity of the just-laid egg may be accurately determined partly by a conical protuberance at the vegetative pole, partly by a clear area (the fading germinal vesicle) at the animal pole. Later the polar bodies at the animal pole give a good landmark. They are also present in the cleavage stages.

The first two furrows are meridional, the blastomeres of the 4-cell stage all being of the same size. Thus we have no predominance of the D-quadrant in the nemertean egg. The third cleavage is dexiotropic, forming four micromeres (1a–1d) and four macromeres (1A–1D), but the micromeres are larger than the macromeres, as Coe (1899) first described (Fig. 1, *A*). At the next (leiotropic) cleavage, the micromeres (the first quartet of micromeres, 1a–1d) are divided into two layers each of four cells ($1a^1$–$1d^1$ and $1a^2$–$1d^2$), and the macromeres bud off a second quartet of micromeres (2a–2d). The macromeres are now called 2A–2D (Fig. 1, *B*).

The use of the words micro- and macromeres at the 8-cell stage is rather confusing, as the micro- are larger than the macromeres. In the following account we prefer to call them the animal and the vegetative cells of the 8-cell stage. Instead of speaking of the two layers derived from the first quartet of micromeres ($1a^1$–$1d^1$, $1a^2$–$1d^2$), of the second quartet of micromeres (2a–2d), and of the macromeres (2A–2D) of the 16-cell stage, we may simply designate the four layers as an_1, an_2, veg_1, and veg_2 (Fig. 1, *B*), as has been done with the four layers in the sea urchin egg (Hörstadius, 1931, 1935). The composition of a larva may then be expressed by a formula, e.g., $an_1 + an_2 + veg_1$ means a larva from which veg_2 has been removed, etc.

The gastrula is still rather opaque, but the fully differentiated larva, the pilidium, is quite transparent. The blastopore forms the mouth, leading into an œsophagus and stomach; there is no intestine or anus (Fig. 2, *A*). One or two days after gastrulation two lappets grow out on the left and right sides of the mouth. The whole surface of the pilidium is ciliated, but the lappets are bordered by a special ciliated band where the cilia are longer, more concentrated, and beat in a characteristic way as compared with the cilia of the rest of the ectoderm. The cells of the ciliated band are more yellowish than the other ectoderm cells. At the animal pole we find an apical organ which is a sense organ, a long flagellum in a thickened pit of the ectoderm. The flagellum is not a single structure but is composed of a bundle of fine threads.

The right and left sides of the pilidium evidently are those which bear the lappets where the lappets are formed. As there is no anus it is difficult to say what corresponds to the dorsal, what to the ventral side. In the gastrula we call that axis dorso-ventral which is perpendicular both to the animal-vegetative and the right-left axes, but in the pilidium the dorso-ventral axis probably, as the ventral side presumably is so short, only forms an acute angle with the egg (animal-vegetative) axis. After metamorphosis the egg axis corresponds to the dorso-ventral axis of the worm.

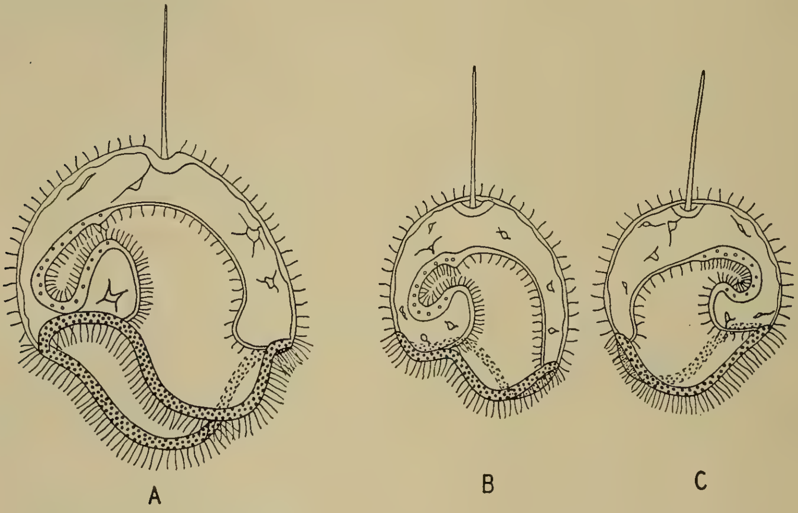

FIG. 2. *A*. Normal pilidium. *B, C*. Pilidia from the isolated two half-blastomeres of one egg.

IV. THE PROSPECTIVE SIGNIFICANCE OF an_1, an_2, veg_1, AND veg_2

The four animal cells of the 8-cell stage were stained by leaning them against a piece of agar. The whole ectoderm down to the ciliated band was blue, and there was also an abundance of blue granules in the band. When the four vegetative cells of the 8-cell stage were stained, the stomach, the œsophagus, the inner sides of the lappets, and the ciliated band were blue. Thus also in this case, we found the ciliated band markedly stained. When the animal cells were stained, the upper, most animal part of the whole band (with one probable exception, see below) contained blue granules, and when the vegetative cells had been stained, the color was restricted more or less to the lower, vegetative part of the band. It is very difficult to trace the limit of the stained area with certainty in *Cerebratulus*, but I think it is evident that the ciliated band is composed of material both from the animal and the vegetative cells.

It was observed in several cases that on one day the young pilidium showed the ectoderm, including the ciliated band, bluish (staining of the animal cells); the next day the stomach too had turned blue. It was noted, however, that the œsophagus and the inner sides of the lappets were still unaffected by the Nile blue. Thus it would appear that we are not dealing with a general diffusion of the stain. It seems probable that at this stage the stomach acts as an excretory organ, as is the case with the digestive tract of turbellarians (Westblad, 1923).

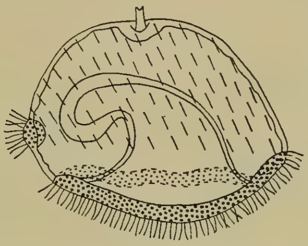

FIG. 3. In the 16-cell stage the four most animal cells, an_1, were isolated, vitally stained and transplanted back on the an_2 cells. The animal fragment was probably rotated 180° at the transplantation: note the little patch of ciliated band too high up on the posterior side. Normally it probably constitutes the most anterior part of the band. Stained area stippled.

Staining of the four veg_2-cells of the 16-cell stage resulted in a blue stomach. Whether a small part of the veg_2-material is or can be used to form a part of the œsophagus I cannot tell,—the limit of the stained area was not sufficiently sharp.

Also in staining the four an_1-cells I had great difficulties in observing the colored line. It seems to me that these four cells form the greater part of the pretrochal ectoderm down to about the equator of the pilidium. Thus an_2 would form the ectoderm on the outside of the lappets and a part of the ciliated band. I have the impression that the an_1-material in the anterior part of the larva goes down to the ciliated band and contributes to the formation of its foremost part, whereas posteriorly it does not reach so far down. This view is supported by the following result. For the purpose of obtaining a sharper limit of the stain, the four an_1 cells were cut off, stained, and replaced. In one case they probably happened to be rotated 180°, because the larva showed a short piece of ciliated band way up from the ciliated band at the posterior end of the pilidium, and the stain border line ran from this isolated piece of band to the band at the anterior end of the larva (Fig. 3). This would indicate that the part of the ciliated band that is normally derived from the an_1-material at the front end of the larva has undergone self-differentiation too high up on the posterior side.

The prospective significance of the four layers thus seems to be as follows: an_1 gives the ectoderm of the upper half of the pilidium, including a very small piece of the ciliated band. an_2 forms the rest of the outside ectoderm (the lappets) and a great part of the ciliated band. veg_1 also contributes to the ciliated band and forms, moreover, the insides of the lappets, and the œsophagus. veg_2 constitutes the material for the stomach and perhaps a small part of the œsophagus as well.

V. THE POSITION OF THE FIRST FURROW

In order to interpret the results of isolating the first two blastomeres, it is necessary to know the position of the first furrow, whether it has a fixed position in relation to the median plane of the larva or may form any angle to this plane. In 34 2-cell stages one blastomere was stained by leaning it against a piece of agar. Owing to the large size of the blastomeres, the stain seldom penetrated the whole blastomere. Thus we did not get a very exact staining, with a sharp border line along a meridian of the egg. The stain rather indicated that side of the blastomere which was opposite the furrow. In 9 of the larvæ the color was too weak or too diffuse. In the other 25 the following parts of the larvæ were found to be blue: dorsal 5, ventral 3, left 6, right 3, dorsal-left 3, dorsal-right 4, ventral-right 1.

The number of cases is small and the method not very exact. Nevertheless, I think these results show that the first furrow is not confined to a certain plane, but probably may have any position in relation to the median plane. Isolating half-blastomeres, we shall thus obtain dorsal and ventral or right and left or oblique meridional halves.

VI. ISOLATION EXPERIMENTS

A. Isolation of Animal and Vegetative Halves of Unfertilized Eggs

Wilson (1903), Zeleny (1904), and Yatsu (1904, 1910*a*) found that fragments from any part of the unfertilized *Cerebratulus* egg may be fertilized and may segment as whole eggs. Zeleny and Yatsu found that fragments obtained during the stages between the fertilization and the completion of the first cleavage show a progressive specification of cleavage factors: just before initiation of the first cleavage the fragments segment as halves. Wilson followed the further development of fragments from unfertilized eggs and found that, if of sufficient size (not smaller than one-quarter), they may produce normally formed dwarf pilidia. According to Yatsu (1910*b*) even an animal half of an egg that has already given off the polar bodies is able to gastrulate and develop into a pilidium.

As the cleavage of fragments has been studied in such detail, I only wanted to repeat the experiments on the localization of gastrulation potencies in fragments of the unfertilized egg. Animal and vegetative fragments were isolated from the beginning of the fading of the germinal vesicle up to the formation of the first polar spindle (as far as the maturation proceeds before fertilization). The eggs used were not very satisfactory for they were the last of the season and the worms had lived for a long time in the ice-box. Eighteen animal and 20 vegetative fragments were fertilized. Five of the animal and 10 of the vegetative fragments died. The remaining 13 animal and 10 vegetative fragments all gastrulated. The best of the vegetative fragments developed into pilidia, whereas the animal ones died before that stage.

Our finding that not only the vegetative, but also all animal fragments gastrulate and that vegetative fragments may form apical organs supports Wilson's results (1903) that fragments from any part of the unfertilized egg are able to produce dwarf pilidia.

B. Isolation of Half-Blastomeres

Charles B. Wilson (1900) was the first to obtain two pilidia from one egg by isolating the half-blastomeres. E. B. Wilson (1903), Zeleny (1904) and Yatsu (1910*a*) found that the isolated half-blastomeres segment, not as wholes, but typically as if the missing blastomere were present, thus a strictly partial cleavage. In the following development, sometimes closed and spherical, sometimes cup-shaped or flat, plate-like forms were observed. All these types of young blastulæ could produce pilidia, but those from the plate-like forms were usually asymmetrical. The best-developed dwarf larvæ seemed to be typical in every respect. But some abnormalities were frequent among the other pilidia. The apical organ was sometimes displaced towards the anterior end (Wilson). However, Wilson never found this organ duplicated in half-larvæ. Zeleny observed no constant defect except possibly in the case of the lappets. But he generally killed his larvæ before the lappets could have developed fully. Yatsu (1910*b*) obtained some perfect pilidia, some with slight abnormalities, among them not infrequently double apical organs. He concludes that bilaterality of egg substances cannot be detected at the 2- or 4-cell stage.

As the segmentation of half-blastomeres has been studied in detail by E. B. Wilson, Zeleny, and Yatsu, I restricted my observations to the differentiated larvæ. The two half-larvæ derived from one egg were reared together in order that the development of the bilateral symmetry in the pairs might be studied. The lappets were formed in four days.

Some larvæ developed into perfect dwarf pilidia. Others were more cylindrical in shape, with a short ciliated band, not forming any lappets. Some looked spherical from the side, namely when the main part of their bodies had the typical form, but the ciliated band was very short, forming only a narrow ring round the mouth. In some cases a lappet was formed on one side only. In many larvæ the apical organ was displaced towards the anterior end, as Wilson (1903) found, but a displacement to the posterior side was also observed. Contrary to Wilson's statement, in several cases I saw the apical organ doubled in the half-larvæ. Not only was the flagellum split up in two, but larvæ with two separated ectodermal pits were found.

If we now turn to the pairs, there were 6 pairs with both larvæ typical, symmetrical, thus each larva with two lappets (Fig. 2, *B*, *C*). The largest group, 13 pairs, is characterized by one typical and one cylindrical larva. In the latter the shape of the œsophagus-stomach generally indicates a bilateral symmetry, which is, however, hardly expressed in the ectoderm. In 6 cases both partners were cylindrical. In 3 pairs one larva was typical, the other irregular and difficult to interpret. One larva had a lappet only on the right side, the partner being cylindrical. In one pair the two pilidia showed supplementary deficiencies: one had a left, the other a right lappet. This is what would be expected if we had a rather definite bilateral organization of the egg in the 2-cell stage. But against this one case were two where both partners had the lappets better developed on the same side, the right.

In the sea urchin, Hörstadius and Wolsky (1936) have demonstrated that the larvæ derived from isolated half-blastomeres, from halves of the 16-cell stage, and even halves of the unfertilized egg, show characteristic differences, according to their origin from the left and right, or dorsal and ventral parts of the egg. In *Cerebratulus* I was not able to refer the half-larvæ to certain parts of the egg. We know that the larvæ obtained may correspond to right and left, or dorsal and ventral, or oblique meridional halves of the egg (*cf.* above). But only in one case of 29 did we find a bilateral asymmetry that would indicate right and left halves. At the same time there were two pairs with the right side better developed in both partners, thus detracting from the value of the supplementary deficiencies in the first-mentioned case. Furthermore, I was not able to detect any features that would incline me to ascribe one partner to the dorsal, the other to the ventral part of the egg. There is no sign of the cylindrical larvæ arising from a dorsal, or ventral fragment. Thus the abnormalities in the cylindrical, rounded, or irregular larvæ do not, as far as I can judge, tell us anything about the origin of these larvæ. Our conclusion, then, would be like that of Yatsu, that the *Cerebratulus* egg in the 2-cell stage is not yet so markedly bilaterally organized that the half-larvæ show deficiencies on the side where material has been removed.

C. Isolation of Quarter-Blastomeres

The quarter-blastomere also undergoes a fractional cleavage analogous to that characteristic of the half-blastomere (Wilson, 1903; Zeleny, 1904). It gives rise to a much larger proportion of abnormal forms, and even at best the larvæ were never normal in every respect (Wilson, 1903; Yatsu, 1910*b*). Zeleny (1904) killed his quarter-larvæ before they were fully differentiated. The apical organ was generally markedly displaced towards the anterior end (Wilson, Yatsu), or it might be lacking (Yatsu). The archenteron, also, could be abnormally formed, displaced towards the posterior end (Wilson).

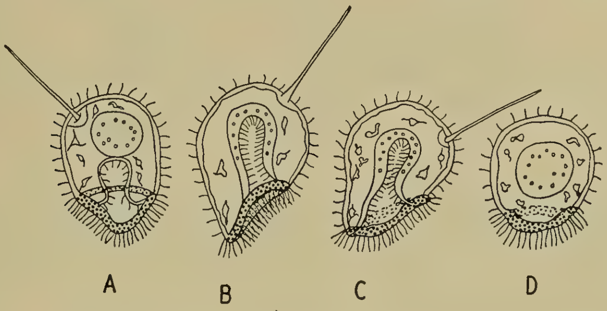

FIG. 4. Four larvæ from the isolated quarter-blastomeres of one egg.

Seven quartets of quarter-blastomeres were isolated, the blastomeres being reared in separate dishes and kept in such order of sequence as in the 4-cell stage, so that we know, for instance, that No. 4 had had Nos. 3 and 1 as neighbors. The larvæ were all more or less irregular (Fig. 4). The apical organ was generally displaced, or might be absent; the archenteron was not always well differentiated. The position of the ciliated band was in most cases more or less oblique. But, as in the half-blastomeres, it was not possible to find any definite relation between these asymmetries and a supposed bilateral organization of the egg.

D. Isolation of the Four Animal and the Four Vegetative Cells of the Eight-Cell Stage

Zeleny (1904) found that larvæ developing from the upper quartet have an apical organ, but no archenteron, those from the lower quartet have an archenteron, but no apical organ, while those from lateral four-cell groups have both apical organ and archenteron. Zeleny drew the conclusion that certain organ-forming materials are definitely separated by the third furrow and that the larvæ from the upper and the lower quartet have not the power of replacing the material lacking. The meridional halves, however, possess both kinds of material. Zeleny preserved his larvæ before they were fully differentiated. Yatsu (1910*b*) isolated 7 animal quartets, six of which gave a blastula with apical organ, with 1 to 3 flagella, and, to judge from the drawings, a ciliated field or band at the opposite pole. Thus no gastrulation occurred. Strange to say, the seventh larva developed into an almost perfect pilidium (Yatsu, 1910*b*, Fig. 18, *D*). Of the 3 vegetative quartets Yatsu mentions two as "defective." The third is an almost normal pilidium (*loc. cit.*, Fig. 18, *I*).

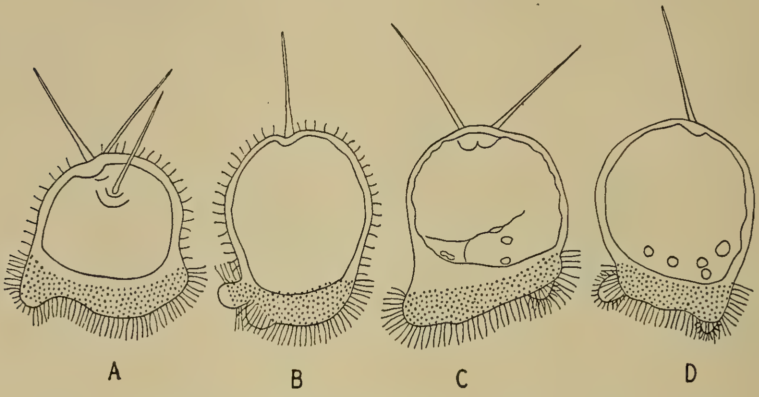

FIG. 5. Larvæ from the isolated four animal cells of the 8-cell stage.

My material numbers 10 pairs of animal and vegetative quartets, and moreover 9 animal and 4 vegetative fragments of the same kind, but of which the corresponding vegetative and animal quartet respectively are missing. None of the 19 animal fragments gastrulated. They gave a blastula with a pretrochal pavement epithelium, a thickened vegetative ciliated band or field, and one, or several, apical organs (Fig. 5). Ten of these blastulæ had but one normal apical organ, 4 had two, and 3 had three flagella. In most cases, perhaps in all, every flagellum had its own ectodermal pit. In one case the apical organ was entirely lacking. One blastula was very peculiar, with seven flagella, three of them on the pretrochal part, the other four growing out from the ciliated band. The shape of the animal larvæ is shown in Fig. 5. It is rather rounded or ovoid, the ciliated band occupying the broader or the narrower end. The "ciliated band" has more the character of a ciliated field of high cells, with here and there some irregular protrusions. This ciliated area has the yellowish color of the ciliated band, and the cilia wave in the same typical way. Thus, on the whole, the animal quartet develops just as it would have done in the normal larva, giving rise to the same kind of tissues: pretrochal ectoderm with apical organ, and ciliated band tissue. A difference lies in the frequent duplication of the apical organ.

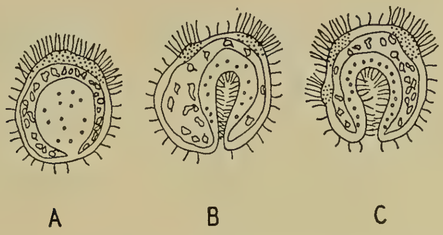

FIG. 6. Larvæ from the isolated four vegetative cells of the 8-cell stage.

The vegetative quartets also (Fig. 6) differentiate in accordance with their prospective significance. An archenteron invaginates, but it evidently corresponds only to the stomach of the pilidium, as it differentiates in the same way, containing the small crystals characteristic of the stomach. The "ectoderm" of the gastrula corresponds to the œsophagus and the insides of the lappets, and at the animal pole of this vegetative fragment we find a ciliated field, presumably derived from the material that normally contributes to the formation of the ciliated band. This field is, however, often divided into two or more ciliated patches of the same kind of tissue as the ciliated band.

All the animal and vegetative fragments thus developed in accordance with their prospective significance. When Yatsu (*cf.* above) found that one animal and one vegetative fragment developed into almost perfect pilidia, he must have isolated two meridional halves instead of one animal and one vegetative. The orientation of the 8-cell stage is not always easy, unless the polar bodies are clearly seen.

E. Isolation of an_1

In two cases Zeleny (1904) successfully separated the four animal cells of the 16-cell stage (an_1) from the twelve lower ($an_2 + veg_1 + veg_2$). The two an_1-larvæ possessed an apical plate but were not reared long enough to show the differentiation in detail.

Twenty-five an_1 were isolated. One did not develop very well. Fourteen disappeared altogether (*cf.* p. 319), and the remaining 10 developed into blastulæ with apical organs (Fig. 7). Five of them had one flagellum, 3 had two flagella, and 2 none. I had the impression that the pit in some cases was missing or not so pronounced as in normal pilidia, but this observation may be erroneous. It may be mentioned that Yatsu (1904), in the uncleaved egg, localized the basis of the apical organ, not in the animal pole, but in a broad zone a little above the equator. Wilson (1903) also observed flagella without ectodermal pit, namely in larvæ from vegetative egg fragments. There is need of further elucidation of the question whether the absence of the ectodermal pit in an_1-larvæ, if this takes place, has any relation to the localization suggested by Yatsu. If so, it would indicate a trace of dependent differentiation.

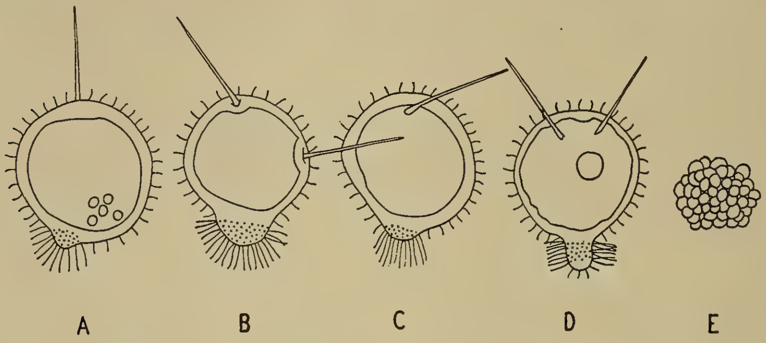

FIG. 7. *A–D.* Larvæ from the isolated four most animal cells of the 16-cell stage: an_1. *E.* Isolated veg_2 (the four most vegetative cells of the 16-cell stage).

The ectoderm of these an_1-blastulæ was ciliated in the usual way. At the pole opposite the apical organ they had small fields of ciliated band tissue (high, yellowish cells). In some cases this field had the form of a protrusion, with the ciliated band like a girdle round it (Fig. 7, *D*). I interpret this to mean that the patch of ciliated band tissue in an_1 corresponds to that part of the normal band which, on the basis of our staining and transplantation experiments, we ascribed to the an_1-cells (See Fig. 3).

F. Isolation of $an_2 + veg_1 + veg_2$

The best one of two fragments of this kind that Zeleny obtained (*cf.* above) formed a ciliated rotating embryo, with a large solid archenteron filling up the cavity of the blastocœl. Neither apical organ nor lappets were present.

Of the five larvæ only one developed really well. In the early gastrula stage the archenteron seemed to fill up the entire blastocœl, as Zeleny found. Also in the early pilidium the archenteron looked very large in comparison to the ectoderm. A day later this was no longer the case to such an extent as one would have expected after removal of such a large part of the presumptive ectoderm (Fig. 8, *A*).

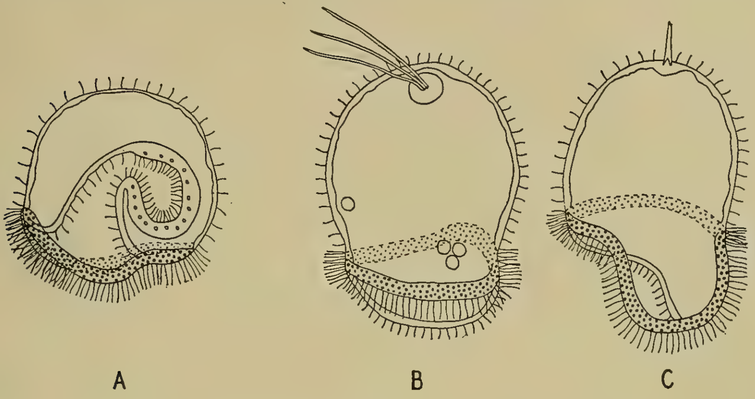

FIG. 8. *A*. $an_2 + veg_1 + veg_2$. *B*, *C*. $an_1 + an_2 + veg_1$.

The larva now appears as a small pilidium, save that the apical organ is missing. The stomach is of normal size. The œsophagus is smaller than normal but probably contains as much material as in the normal pilidium, as its wall is thicker. The ciliated band and the pretrochal ectoderm are too small, which is easily understood in that the larger part of the ectoderm has been removed. That, in spite of this, the ectoderm does not look still narrower is probably due to an extreme stretching and thinning of this material.

G. Isolation of veg_2

This region constitutes the material for the stomach (perhaps also a small part of the œsophagus). When isolated it does not live long. On the first day after fertilization these fragments were generally all dead. Those which lived the longest formed a mass of cells, as seen in Fig. 7, *E*. Only in one case were some cilia developed, indicating an initial differentiation.

H. Isolation of $an_1 + an_2 + veg_1$

Only three fragments of this type were studied, but they all developed in a similar way. They formed, much as the isolated four animal cells of the 8-cell stage, a blastula with an apical organ and a ciliated band (Fig. 8, *B*, *C*). Thus no real deep gastrulation took place, although the vegetative end of the larva at one stage may be a little curved inwards, as in a normal larva just at the beginning of the gastrulation. This part later differentiated into an epithelium which mostly looked like ectoderm, but perhaps was a little more densely ciliated. In the larva from the upper quartet of the 8-cell stage the "ciliated band" rather was a ciliated field of high cells, occupying the lower part of the larva (Fig. 5). Our present larvæ differ in the respect that we here have a real band, surrounding the ectoderm-like area just mentioned, which in turn must correspond to the inside of the lappets and the œsophagus. In one of the larvæ this area first curved inwards and was later turned out again (Fig. 8, *B*), whereas in the other two it remained as a very shallow invagination; the ectoderm formed like a lappet on one side (Fig. 8, *C*). Thus also a larva from which veg_2 has been removed seems to develop in accordance with the prospective significance of the material.

I. Isolation of $an_2 + veg_1$

The same holds for the fragments $an_2 + veg_1$. A great many larvæ disappeared. The 9 surviving ones differentiated, although varying in shape, into blastulæ with a broad, ciliated band around the equator (Fig. 9). As the ectoderm does not expand, the ciliated band is very thick and broad. The pretrochal, animal epithelium which corresponds to the thin ectoderm formed by an_2 is generally thinner than the vegetative epithelium of the band (corresponding to the inside of the lappets and the œsophagus). Some larvæ are more or less massive, and irregular in shape (Fig. 9, *C*, *D*).

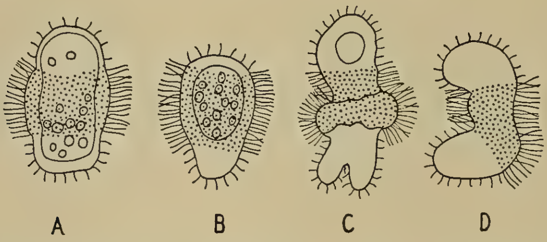

FIG. 9. Larvæ from the two middle layers of the 16-cell stage: $an_2 + veg_1$.

VII. Transplantation Experiments

A. $an_1 + veg_2$

The most animal and the most vegetative of the cell layers of the 16-cell stage were fused for the purpose of studying whether organs that normally arise from the middle part of the egg could be formed by regulation. The larvæ gastrulated, the veg_2-material being invaginated. The ectoderm appears identical with that of the isolated an_1, having an apical organ and a small patch of ciliated band tissue (*cf.* Figs. 10 and 7). In some cases the archenteron differentiated into just a stomach, very characteristic with its crystals in the wall. But in other cases I also obtained what looked like a small part of an œsophagus (Fig. 10, *C*). This might be a structure formed by regulation, but, as pointed out on p. 321, it is possible that veg_2 normally also forms a part of the œsophagus. I am not sure that the third furrow always lies at the same level. I have the impression that the differences in size between the animal and the vegetative cells of the 8-cell stage may vary. As a consequence probably the cleavage plane veg_1–veg_2 also may vary in relation to the limit between the presumptive œsophagus and presumptive stomach. This would explain the slightly different results (Fig. 10, *A–C*).

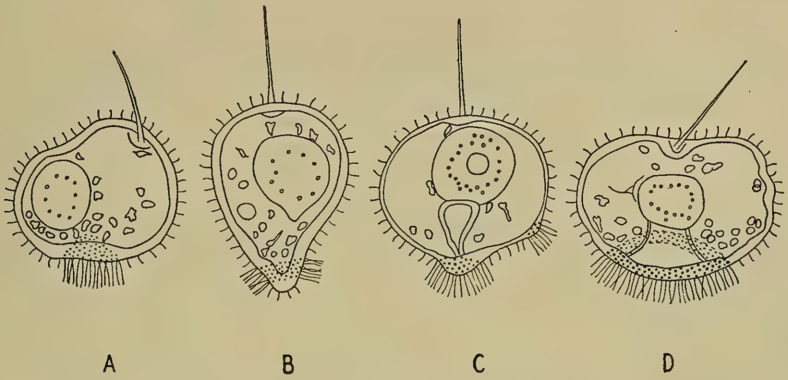

FIG. 10. *A–C.* Larvæ composed of the most animal and the most vegetative layers of the 16-cell stage: $an_1 + veg_2$. *D.* $an_1 + \frac{an_2}{2} + veg_2$.

B. $an_1 + \frac{an_2}{2} + veg_2$

In three cases veg_2 was added to an_1 + 2 an_2-cells. This increase in ectoderm and ciliated band material gave a larva of essentially the same type as $an_1 + veg_2$, but with a larger ectoderm and a larger amount of ciliated band tissue, which now forms a complete ring (Fig. 10, *D*).

C. Fusion of an Animal and a Meridional Fragment of the Eight-Cell Stage

The problem is whether the entoderm material of a meridional fragment of the 8-cell stage can bring about any entodermization of adjacent presumptive ectoderm from an animal fragment, and whether a regulation will take place also regarding other organs (apical organ, ciliated band), so that a harmonic individual arises. The animal fragment of the 8-cell stage was vitally stained before transplantation. At the fusion the polarity of this fragment stands at right angles to that of the meridional one (Fig. 11, *A*).

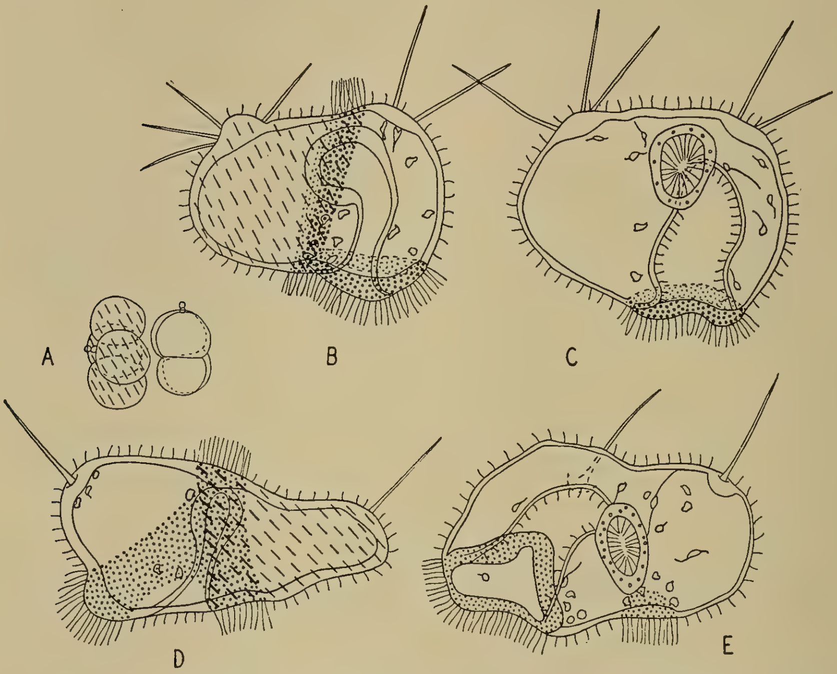

FIG. 11. *A*. Fusion of a meridional half and a vitally stained (stippled) animal half of the 8-cell stage. *B*, *D*. The larvæ have differentiated conformably to their prospective significance. *C*, *E*. Commencing regulation by redifferentiation.

The 5 larvæ all gave the same result (Fig. 11, *B*, *D*). Each component differentiated in the same way as it would have done normally; there seemed to be no interaction between them whatsoever. At gastrulation none of the adjacent blue animal material was invaginated. The meridional partner developed an apical organ at its animal pole and half of a ciliated band, embracing the mouth like a horseshoe. The animal component clearly has a polarity at right angles to that of the meridional one. The apical organ of the animal fragment is situated at its animal pole; its ciliated band forms a girdle round its base, at right angles to the horseshoe of the meridional component. In the larva, Fig. 11, *D*, the apical organ of the meridional fragment is not situated close to the stained ciliated band. The position of the two ciliated bands gives the explanation. They are not at right angles to each other. The fusion of the two components has occurred obliquely.

Thus both halves differentiated according to their prospective significance. It is now highly interesting to note that, in spite of the lack of interaction in the period of embryological differentiation, we find a regulative interaction in the following development. Several days after the differentiation of the organs of the pilidium, the ciliated band of the animal component gradually disappeared. Only a short part of it remained to form, together with the horseshoe of the meridional part, a complete ciliated ring (Fig. 11, *C*; *cf.* also *E*). Thus we here witness a step towards the individualization of this heterogeneous larva by means of redifferentiation. The larvæ did not live long enough to show any further changes.

VIII. Conclusions and Discussion

By means of vital staining the prospective significance of the animal and the vegetative cells of the 8-cell stage, and of the four cell layers an_1, an_2, veg_1, and veg_2 of the 16-cell stage, was studied. The isolation experiments showed that, on the whole, the fragments isolated by cuts vertical to the egg-axis differentiated in accordance with the prospective significance of such parts interpreted through the presence of different organs, e.g., apical organ, ectoderm pavement epithelium, ciliated band, epithelium of the inside of the lappets, œsophagus, and stomach. There is some doubt, however, as to whether all fragments differentiate in every detail according to their prospective significance, as the latter could not be determined with accuracy in all cases. The staining experiment was not quite conclusive as to the fate of an_1, but the transplantation experiment (Fig. 3), too, indicates that an_1 normally forms a piece of the ciliated band, as the isolated fragment always does (Fig. 7). It is more uncertain whether veg_2 normally contributes to the œsophagus. We must thus leave open the question whether the small œsophagus in some of the larvæ $an_1 + veg_2$ (Fig. 10) was formed from presumptive œsophagus or by regulation. The rather extensive ectoderm in the larva $an_2 + veg_1 + veg_2$ may result from an unusual stretching of the material. The mosaic development of the larvæ in Fig. 11 supports the view that the apparent deviations from the prospective significance in the differentiation of the cases just mentioned are more apparent than real.

It was suggested as possible that the apical organ is not definitely determined in an_1. An interesting feature is the appearance of several apical organs in fragments (not only is the flagellum subdivided but there may also be several ectodermal pits). Is that a sign that the organ was not determined at the time of operation? Not necessarily, as it may, on the contrary, indicate a mosaic differentiation. If the apical organ is normally formed from all four quadrants, a slight relative change of position of the animal parts of the blastomeres at or after the operation may cause the parts of the presumptive apical organ to become separated by other ectoderm. In my experiments I never got more than two apical organs in half-larvæ, only one in the quarter-larvæ, and, with one exception, not more than two or three in animal fragments, thus never more than the number of quadrants present. On the other hand, the exception, an animal fragment with seven separate flagella (p. 327) seems to show, as well as the possible lack of ectodermal pit in an_1-larvæ (see p. 328), that the determination of the apical organs in fragments is more complicated. The problem must be left unsolved.

The scope of this investigation was to compare an egg with mosaic development with that of the sea urchin. An animal fragment of the sea urchin egg (an_1, or $an_1 + an_2$ of so-called equatorial eggs (Hörstadius, 1935, p. 309)) forms less organs (no ciliated band, no stomodæum) than it would have done in normal development, whereas a vegetative half, by regulation, often forms more organs than the same material would normally have yielded (the vegetative halves may have apical tuft, mouth, oral arms). An isolated veg_2-layer develops into a larva with both more animal (ectoderm) and more vegetative differentiations (skeleton) (*loc. cit.*, p. 423) than it would have given rise to in normal development (archenteron). Another way of demonstrating the changes of differentiation that may occur in the sea urchin is to add a vegetative fragment to an animal fragment, e.g., to add the four micromeres to an_1 or to an animal half ($an_1 + an_2$). As a result of an interaction between the animal and the vegetative qualities a complete dwarf pluteus develops (*loc. cit.*, p. 330).

Our experiments with *Cerebratulus* have shown that there is probably no change in differentiation in animal and vegetative fragments of cleavage stages. If any changes take place they are so slight that we have not been able to detect them with certainty. At all events, there is no regulation to compare with that of a vegetative half, or a veg_2, in the sea urchin, and, in the *Cerebratulus* fragments, there is no failure of some organs to appear (in comparison to the prospective significance) as in the isolated an_1 or $an_1 + an_2$ of the sea urchin. We have several directly comparable experiments which show this very clearly. In the sea urchin it is possible to obtain two plutei from one egg, after cutting at right angles to the egg-axis, i.e., if we cut twice and put the two polar parts together (Hörstadius, 1936*a*). The middle part will thus give more animal and more vegetative differentiations than it would have done normally, the animal and vegetative fragments (an_1 + the micromeres, or $an_1 + an_2$ + the micromeres) will, by regulation, form the organs characteristic of the excised middle part of the egg (archenteron, etc.). The equivalents to these experiments are our isolations of $an_2 + veg_1$ (Fig. 9) and fusion of $an_1 + veg_2$ (Fig. 10) in *Cerebratulus*. As far as we can judge, these larvæ show mosaic development. The second experiment implies the adding of an animal half to a meridional half, at right angles to each other as regards polarity. This larva differentiates in the sea urchin into a perfect pluteus. The animal half becomes completely incorporated: a part of it is entodermized, now taking part in the formation of the archenteron, and the ectoderm, too, acts in all details (ciliated band, stomodæum) as a part of the new individual (Hörstadius, 1928, 1935). In *Cerebratulus* this is not true (Fig. 11). We find a strict mosaic development, without any trace of entodermization or accommodation of the ectoderm of the animal fragment to that of the meridional half.

I recall the fact that the ciliated band of the animal component of these larvæ may disappear. We witness an initial change of the heterogeneous larva into a more harmonic individual. It has already been pointed out (p. 333) that this change is brought about by a redifferentiation after the embryological differentiation is already complete. In this connection we must mention a similar observation by Yatsu (1910*b*). Whereas an apical organ is not formed in larvæ which in the cleavage stages were deprived of their most animal part, Yatsu found that the apical organ regenerated, when it was removed from late gastrulæ or young pilidia.

We have to remember that the mosaic development of animal and vegetative parts mentioned above concerns the layers of the 8- and 16-cell stages. Our isolation of animal and vegetative halves prior to fertilization confirms the results of Wilson (1903) and Yatsu (1910*b*), —that any fragment of the unfertilized egg may develop into a pilidium. Thus the transition from a regulation to a mosaic type takes place between the beginning of maturation (before fertilization, *cf.* p. 322) and the 8-cell stage. Yatsu (1910*b*) observed that an animal half of even a mature egg could form a pilidium. Removal of the most animal part of the egg in the first cleavage of the two blastomeres of the 2-cell stage had no effect, but when the most vegetative material was cut off, the archenteron was missing or too small (Yatsu). Thus at that time the localization of the presumptive entoderm seems to have advanced a great deal. When 2-cell stages were compressed so that the second furrow came to lie equatorially, instead of meridionally, and the two animal cells were separated from the vegetative, the former did not gastrulate, whereas the vegetative gastrulated but formed no apical organ. Thus already at the 4-cell stage we seem to have exactly the same animal-vegetative localization as in the 8-cell stage.

Wilson (1903) and Zeleny (1904) have assigned the location of the entoderm material to the vegetative part of the egg and the determination of the apical organ to the rearrangement of material at the breaking down of the germinal vesicle. Yatsu (1904) observed, in sections, that a segregation of egg material does actually take place at that period, the yolk accumulating in the lower hemisphere while the clear and more finely granulated protoplasm collects at the animal pole of the egg. Some of the results presented by Yatsu as to the time at which determination has taken place are contradictory. Further investigations are desirable as to how much the determination becomes fixed along with the visible rearrangement of materials, and how much is a progressive process going on after the completion of this rearrangement, and hence a process of a different character.

The rearing of fragments of blastulæ has given startling results. It would seem as if these fragments were richer in potencies than fragments of cleavage stages. Wilson (1903), Zeleny (1904) and Yatsu (1910*b*) found that animal halves of blastulae could gastrulate, although the archenteron was generally smaller than that of the corresponding vegetative fragment. This result is in conflict with everything we know of the progressive embryological determination which leads to gradual restriction of potencies in every zone. (We recall the fact that the regeneration of an apical organ in the young pilidium and the redifferentiation in our larvæ meridional half + animal half took place only after the completion of the embryological differentiation—*cf.* p. 335.) Wilson is inclined to explain at least some of his results on the grounds of oblique sections. In view of this, Zeleny (1904) took special care in determining the orientation, but nevertheless he found in his two cases that the blastula fragments had greater regulative power than those of the 8- and 16-cell stages. One of Yatsu's animal fragments had a small gut, and the vegetative was provided with an apical organ. Until these results have been confirmed by means of very careful experiments, I am, like Wilson, inclined to explain them on the grounds of oblique sections. It is much more difficult to cut a blastula in a desired plane, than a cleavage stage. In the latter you follow a furrow all through the egg. Moreover, if you start cutting a blastula equatorially with the correct orientation, the result may be oblique halves, for the knife may pass obliquely in animal or vegetative direction through the egg. This can only be detected by a careful examination of the fragments from all sides. The variability of the results on blastulæ, and the uniformity of those on 8- and 16-cell stages speak in favor of this assumption. As the fragments of the uncleaved egg are more or less equipotent, oblique cutting is most readily detected in the blastula stage.

If we turn to the fragments in which all the layers are present in the same proportion as in the egg (isolated half and quarter-blastomeres), we find that they are able to develop into more or less typical pilidia. All the organs are present, but the larvæ may be more or less asymmetrical, especially the quarter-larvæ. On the other hand, several pairs of half-larvæ showed a perfect bilateral symmetry (Fig. 2, *B*, *C*). These pairs probably correspond to both right-left, dorsal-ventral, and obliquely separated meridional halves (*cf.* p. 322). Furthermore, the pairs with asymmetrical larvæ did not show from which parts of the egg the halves originated. This seems to indicate that the bilateral symmetry is not fixed at an early cleavage stage when the animal-vegetative layers are already determined. In this respect a comparison with the sea urchin is of great interest. It has not been possible to study experimentally the unripe egg of the sea urchin. In the mature egg, before fertilization, we find not only an animal-vegetative segregation of presumptive ecto- and entoderm, but also slight traces of a bilateral organization. These traces are more marked in the cleavage stages. Right and left halves show complementary deficiencies of the skeleton on the cut side. Ventral halves develop their ventral side faster and better than dorsal halves. It is very interesting that the dorso-ventral axis in the dorsal halves is inverted (Hörstadius and Wolsky, 1936). I regret that time did not allow me to study in a similar way (by staining the cut side of the two isolated half-blastomeres) the relation of the median planes of the half-larvæ to that of the egg of *Cerebratulus*.

Thus the animal-vegetative determination in *Cerebratulus* seems to take place between fertilization,—at the beginning of maturation,—and an early cleavage stage. In the sea urchin we find a localization of presumptive ecto- and entoderm already in the mature, unfertilized egg but the same degree of determination is not reached as in the 8-cell stage of *Cerebratulus* until much later; in the case of some organs not until the beginning of gastrulation (Hörstadius, 1936*b*). On the other hand, the unfertilized, but mature sea urchin egg already seems to have a bilateral organization, which can be traced in meridional fragments, whereas a bilateral organization of the *Cerebratulus* egg is not possible to detect, even in the early cleavage stage. It has many times been pointed out in the literature that there is no fundamental difference between the mosaic and the regulation eggs. In some eggs the determination sets in at an earlier stage than in others. This relative displacement in time is strikingly illustrated in these cases. In the *Cerebratulus* egg the animal-vegetative determination is accomplished much earlier; the determination of the bilateral symmetry later than in the sea urchin egg.

The formation of mesenchyme has not yet been mentioned. It would have been of great interest to determine whether, in fragments, mesenchyme can be budded off from other parts of the egg than those under normal conditions. In the figures, mesenchyme cells have been drawn in those larvæ in which they were clearly seen. In many isolated fragments we find cells in the blastocœl which are not real mesenchyme cells, but pathological. Those are more rounded, and often larger than the true mesenchyme cells. We find typical mesenchyme cells in the half- and quarter-larvæ (Figs. 2 and 4), in the isolated vegetative cells of the 8-cell stage (Fig. 6), in $\mathrm{an}_1 + \mathrm{veg}_2$ (Fig. 10), and in the meridional half + the animal fragment (Fig. 11). Many of the an_1 (Fig. 7, *B*, *C*) and the $\mathrm{an}_1 + \mathrm{an}_2$-larvæ (Fig. 5, *A*, *B*) were empty, others had some cells of the pathological type in the blastocœl (Fig. 7, *A*, *D*, 5, *C*, *D*). Only in one case was just one single cell observed which looked like a true mesenchyme cell (Fig. 5, *C*). The $\mathrm{an}_2 + \mathrm{veg}_1$-larvæ had some cells in their interior, but I could not determine their nature. To summarize, the larvæ with veg_2 present show typical mesenchyme cells (Figs. 2, 4, 6, 8*A* cells not drawn, 10, 11). The animal fragments (Figs. 5 and 7) have with one exception no typical mesenchyme cells. As regards the rôle of veg_1, we cannot say anything with certainty. The character of the cells in Fig. 9 ($\mathrm{an}_2 + \mathrm{veg}_1$) is unknown, and as to the larvæ $\mathrm{an}_1 + \mathrm{an}_2 + \mathrm{veg}_1$ (Fig. 8, *B*, *C*), I have no records regarding the mesenchyme cells. Even with more detailed observations on the occurrence of the mesenchyme in fragments, it would be difficult to state whether the prospective potency as regards mesenchyme formation exceeds the prospective significance, as observations on normal mesenchyme and mesoderm formation are varied and contradictory. Coe (1899) traces the mesenchyme to the divisions of a large posterior pole cell, as in annelids, and to some of the entoderm cells. Charles B. Wilson (1900) speaks of micro- and macromesencytes, both derived from large entoderm cells close to the ectoderm. E. B. Wilson (1903) describes two symmetrically placed mesoblast cells which, just before invagination, pass into the cleavage cavity near one end of the embryo, and from them smaller mesenchyme cells are budded forth, without, however, giving rise to definite mesoblast bands, as in the annelid embryo. E. B. Wilson finds it probable that the first two mesoblast cells do not arise from a single cell. All these statements refer to the genus *Cerebratulus*. Nusbaum and Oxner (1913) compare the observations of previous investigators with their own and come to the conclusion that the mesenchyme in the genus *Lineus* has a double origin: "(1) Aus Mesoblastzellen, die sich sehr früh aus den Mikromeren des vierten Quartetts differentieren und (2) aus nachträglich schon im Gastrulastadium aus dem Entoderm sich abtrennenden Zellen." According to Nusbaum and Oxner (1913) the micromere 4d in *Lineus* divides into two teloblasts (Urmesodermzellen), whereas Lebedinsky (1897), in *Tetrastemma* and *Drepanophorus*, speaks of four mother cells for four mesoblast bands. In *Malacobdella* Hammarsten (1918) also traces the mesoderm to four mother cells, 2a–2d. Thus the observations diverge widely, even within one genus, and even within one species. A renewed study of the mesenchyme formation in *Cerebratulus*, combined with isolation experiments, would be of great value. One problem is whether veg_1 normally and in fragments buds forth any mesenchyme. As half-larvæ (which may be right-left as well as dorsal-ventral halves) and quarter-larvæ always have mesenchyme cells, a second problem is whether the mesenchyme normally arises in all four quadrants of the egg, or the mesenchyme in some of the meridional fragments is formed by regulation. It may be added that the mesenchyme of the pilidium corresponds to both the larval mesenchyme (ectomesoderm) and the entomesoderm of the trochophore, as a part of the mesenchyme cells in the pilidium preserve an indifferent character until the formation of the worm.

It would lead us too far to consider here all experiments on other eggs with spiral cleavage. A full review of the literature on annelids and mollusks has been given by Schleip (1929); see also Huxley and de Beer (1934, Chapter V). The main difference between the nemertean egg and those of the other groups is the equality of the four quadrants of the egg in the former. In the uncleaved eggs of annelids and mollusks we are dealing with specific substances, more or less visible, which are of an "organ-forming" nature, the pole-plasms. They are precociously chemo-differentiated (Huxley and de Beer) to varying degrees in different species, and, moreover, the time at which they become specifically localized varies. Very often a polar lobe is formed temporarily during the first cleavage divisions. Removal of this lobe leads to an absence of the apical organ and to a defective post-trochal region; no somatoblasts will be formed. Because of this the isolated quarter-blastomeres develop in a very different way. Only the D-quadrant—the one that has the polar lobe—can produce somatoblasts (which give rise to the ectodermal and mesodermal (entomesoderm) germ-bands. The A-, B-, and C-blastomeres cannot develop into complete larvæ. They may have larval mesenchyme (ectomesoderm), but are devoid of entomesoderm. As regards the apical organ, the conditions are, however, not so uniform. In *Patella*, for example, (Wilson, 1904*b*) all four quadrants acquire an apical organ, in *Dentalium* (Wilson, 1904*a*) only the D-quadrant, whereas in *Sabellaria* (Hatt, 1932) the factor determining the apical organ goes to the C-quadrant.

In mosaic development we thus find (at varying times) precociously differentiated substances distributed to particular regions of the egg, and in varying degrees by different forms, and subsequently isolated by the cell divisions. If the vegetative pole-plasm be equally distributed to the first two cells (*Tubifex*, by heat or deprivation of oxygen, Penners, 1924; *Chætopterus*, *Nereis*, and *Cumingia* by compression, high or low temperature, centrifuging, or anaerobiosis, Titlebaum, 1928, Tyler, 1930) double monsters are formed, or the blastomeres may, if isolated, give rise to more or less complete larvæ. In isolated normal half-blastomeres we thus find a self-differentiation in AB, on the one hand, differing from that of CD on the other, but when the vegetative pole-plasm is evenly distributed, both are capable of producing all organs. An interesting problem still remains unsolved. The isolated normal blastomeres differentiate as fragments, but would the same blastomeres behave in the same way if placed in contact with each other atypically? Although the "organ-forming" substances are now separated by cell-walls, could any kind of interaction be detected, any kind of change of the prospective significance of the material be brought about? Penners (1926, 1934) obtained, after killing teloblasts of *Tubifex* at different stages, a development in many respects of mosaic character, but also detected a certain dependence of the ectodermal and mesodermal components of the germ-bands on each other, and on the entoderm of the germ-bands, etc.

In *Cerebratulus* we also have an early rearrangement of substances, namely at the time of the breakdown of the germinal vesicle. But these substances are not, as far as we know, unevenly distributed to the quadrants. As has been pointed out above, we do not yet know with certainty or in detail at what time the progressive animal-vegetative determination is accomplished. The potencies of the blastula are still particularly obscure. Nor do we know how much of that determination is due to a rearrangement of substances, or to another metabolic process. A close study of mesenchyme formation in *Cerebratulus*, both in normal development and in animal-vegetative and meridional fragments, would be of special interest in rendering possible a comparison with the conditions in annelids and mollusks.

IX. Summary

1. The prospective significance of the animal and the vegetative cells of the 8-cell stage, and of the layers an_1, an_2, veg_1, and veg_2 of the 16-cell stage (Fig. 1) was studied. an_1 forms the greater part of the pretrochal ectoderm, including (probably) the most anterior part of the ciliated band (Fig. 3). an_2 gives rise to the rest of the pretrochal ectoderm, and a great part of the ciliated band. veg_1 also partakes in the formation of the ciliated band, and, moreover, differentiates into œsophagus and the insides of the lappets. veg_2 corresponds to the stomach. The boundary between the layers could not, however, always be determined with complete accuracy.

2. The first furrow may form any angle to the median plane of the larva.

3. The two isolated half-blastomeres from one egg may develop into pilidia with perfect bilateral symmetry (Fig. 2 *B*, *C*). When the isolated half- and quarter-blastomeres are less typical, their abnormalities do not show from which part of the egg the dwarf larvæ come. The bilateral symmetry is not determined in the early cleavage stage.

4. Animal and vegetative fragments differentiate, as far as we can judge from comparison with our results concerning the prospective significance, in the same way as they would have done in normal development: the animal (Fig. 5), and the vegetative (Fig. 6) cells of the 8-cell stage; an_1 (Fig. 7, *A–D*); veg_2 (Fig. 7, *E*); $an_2 + veg_1 + veg_2$ (Fig. 8, *A*); $an_1 + an_2 + veg_1$ (Fig. 8, *B*, *C*); $an_2 + veg_1$ (Fig. 9).

5. Larvæ composed of $an_1 + veg_2$ did not form any of the organs normally derived from the excised middle part of the egg (Fig. 10). Fusion of an animal with a meridional half of the 8-cell stage also showed a complete mosaic development (Fig. 11).

6. The results are discussed and compared with those with sea urchins, annelids and mollusks. The desirability of further investigations is emphasized.

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PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

JULY 6, 1937

Parthenogenetic merogony in the Naples sea-urchins. Ethel Browne Harvey.

Parthenogenetic merogony, or development of non-nucleate parts of eggs which have been artificially activated, has been studied in the four species of sea-urchin commonly occurring at Naples. The non-nucleate fractions were obtained, as previously, by breaking the eggs apart with centrifugal force.

In *Arbacia pustulosa,* which is similar to the *Arbacia* at Woods Hole, asters and cleavages occur in the non-nucleate half; when activated, similar to what has been described for *Arbacia punctulata.*

In *Parechinus* (*Echinus*) *microtuberculatus* and *Paracentrotus* (*Strongylocentrotus*) *lividus,* the fertilization membrane in activated non-nucleate halves is well separated from the cell surface, just as in normally fertilized whole eggs of these species. A large monaster is formed, an amphiaster and there are one or two cleavages. The cleavage planes frequently disappear and some time afterward the egg breaks up into a number of pieces. These become progressively smaller and more numerous until what resembles a blastula is formed. This breaks through the fertilization membrane, but no further development has been observed. The non-nucleate halves of *Sphaerechinus granularis* can also be activated and they break up into small pieces in a similar way.

It might appear that this breaking up of the egg is a form of degeneration or cytolysis. But the same spontaneous breaking up of the cell has been observed also in normal whole nucleate eggs artificially activated. These sometimes gave rise to typical blastulae which became free swimming and looked normal in every respect.

It has also been found that by treating the immature egg with parthenogenetic agents, there is formed a very definite layer on the periphery of the cell similar to the ectoplasmic layer in the fertilized or activated mature egg. This is formed both on the half containing the germinal vesicle and the heavier half separated from it by centrifugal force. Another evidence of activation is the failure to respond to sperm by the formation of blisters on the surface. The treated, immature egg, after some time, pinches off a small piece, always at the part most distant from the germinal vesicle in elongated eggs. Later there are two, three or more pieces and then a great number.

A recent critical examination of prepared slides of the parthenogenetic merogones of *Arbacia punctulata* shows the presence of asters, usually in pairs, but no chromosomes. The Feulgen reaction, in the procedure of which I was assisted by Dr. Jean Brachet, is entirely negative. The parthenogenetic merogones show no red-staining nuclear material, whereas the fertilized merogones which were used as control material, show it very clearly.

Some oxidative properties of isolated amphibian germinal vesicles. Jean Brachet.

Amphibian germinal vesicles isolated from full-grown oöcytes in a saline solution absorb oxygen and eliminate carbon dioxide during several hours; the oxygen consumption was measured by means of a modified Garard-Hartline micro-respirometer using *Triturus pyrrhogaster* as material while the CO_2 excretion of *Rana fusca* nuclei was estimated by a titrimetric micro-method. The QO_2 $\frac{(\text{cu. mm. } O_2)}{\text{grams wet weight} \times \text{hour}}$ of the isolated nuclei (the approximate wet weight being calculated from the volume and the density) is about 13 while the full-grown oöcytes have a QO_2 of 37. The respiration of one single isolated nucleus is only 1–1.5 per cent of the amount of gas exchanged by one full-grown oöcyte deprived of its follicular epithelium. Removal of the germinal vesicle does not affect markedly the CO_2 elimination of full-grown oöcytes during several hours. Addition to the nuclei of glucose or of cytoplasm removed from the oöcytes with a micropipette does not increase their respiration. Presence or absence of Ca ions (which, according to W. R. Duryee, greatly affect the physical properties of the germinal vesicle) does not affect appreciably the metabolic rate of the isolated nuclei.

Influence of temperature and other agents on the respiration and development of marine eggs. Albert Tyler. (Most of the data given in this report appear in an article on p. 261 of this issue of the Biological Bulletin under the title, " On the energetics of differentiation, VI." The report has also been abstracted in the Collecting Net of July 10.)

July 13

Effects of fatigue due to muscular exercise on the Purkinje cells of the cerebellum of mice at various ages. Warren Andrew.

The problem of morphological changes in nerve cells as the result of functional activity has been studied by a number of investigators. Nevertheless, the question still remains open not only as to what changes occur due to activity but as to whether any such changes do occur.

Among the factors to be taken into account in such work is that of age. The present experimental work is based on earlier work in which the Purkinje cells of mice and rats were studied from the time of their differentiation up to and including extreme senility of the individual.

Sixteen black mice were used in the experimental work on fatigue. " Fatigue " means complete exhaustion brought about by running in a motor-driven rotary cage. For each fatigued animal, a control animal of the same brood was killed at the same time and the tissues from the two animals carried through the technical processes together.

The animals represent a range of ages including pairs of 23 days, 25 days, 43 days, 46 days, 98 days, 101 days, 746 days, and two animals with marked signs of senility—of 744 and 746 days, killed without fatiguing. In each case 100 cells were examined.

The major conclusions to be drawn from the present work are: 1. There are morphological changes in nerve cells as the result of fatigue carried to exhaustion, consisting primarily in a loss of Nissl material, an increased basophilic property of the nucleus, and an increased average cell size. 2. In senile animals there is also a loss of Nissl substance and an increase in the basophilic properties of the nucelus. The binucleate condition of the Purkinje cell is a phenomenon of senility. The differences between the Purkinje cells of senile and of young animals are far more marked than are those between exhausted and fresh animals of the same age.

Localization in the oculomotor nuclei of the goldfish. Zareh Hadidian, Milton S. Dunn and Roland Walker.

The position of cells innervating the individual eye muscles was studied to see whether there is any pattern in the oculomotor nuclei which might be correlated with the type of eye coördination, which differs from that in animals with binocular vision.

The method was a study of chromatolysis in the oculomotor cell bodies after cutting a peripheral nerve or its muscle, since these operations gave similar results. In normal animals there was a negligible proportion of cells showing advanced degeneration, while in animals with both eyes removed practically all the cells showed chromatolysis. There were no changes in the nuclei studied after removal of the contents of an eyeball.

When the contents of one orbit were removed, thus insuring complete damage to all fibers of III and IV on that side, about half of all the oculomotor and trochlear cells were altered. Chromatolysis in the trochlear nucleus was contralateral, while the degenerate oculomotor cells were about 70 per cent homolateral, the contralateral ones being mostly in the ventromedial portion.

Chromatolysis after damage to the inferior oblique muscle or its nerve was about 80 per cent homolateral, with a slight tendency toward ventromedial localization. For the anterior rectus degeneration was about 60 per cent homolateral with no localization. The inferior rectus showed about 85 per cent homolateral degeneration with definite localization in the dorsolateral nucleus anteriorly, changing gradually toward the ventromedial nucleus posteriorly. The superior rectus had only about 20 per cent homolateral cells, and the contralateral group was localized in the ventromedial nucleus.

A consideration of these results leads to the conclusion that since there is poor definition of cell groups, and scattering of cells for any one muscle throughout the nucleus, any merely anatomical studies of oculomotor localization are inadequate for the understanding of the type of eye coördination in the goldfish.

Some new observations on the secretory activity of neurones. E. Scharrer. (Followed by Demonstration.)

Nerve cells having more or less the appearance of secretory cells have a wide distribution. Among the invertebrates they have been found in annelids, molluscs, crustaceans and insects; among the vertebrates, in the diencephalon of selachians, teleosts, amphibia, reptiles and mammals, including man. In the case of the bony fishes, gland-nerve cells have also been found in the nucleus of the nervus terminalis, in the midbrain and in the caudal region of the spinal cord; the latter being especially well developed in selachians (see Speidel, 1919, 1922). All stages can be found, from typical nerve cells containing only a few granules in the cytoplasma to cells with a spectacular formation and storage of droplets of a colloid-like substance. There are even cases, such as that of the Mediterranean fish *Cristiceps,* where the nerve cells in the so-called "diencephalic gland" are transformed into gland cells lacking any nervous character. A marked nuclear polymorphism is also typical for many gland-nerve cells and pericellular as well as endocellular blood capillaries are often observed in the secretory diencephalic nuclei of vertebrates.

Observations of this kind, even when based on such extensive material, would not suffice to prove the glandular function of the cells in question. It must be demonstrated that there is a functional cycle in the production of the colloid material by the nerve cells in the neuro-secretory regions of the nervous system. This has been done with the diencephalic gland (nucleus praeopticus) of the American toad (*Bufo americanus*). Sections through this gland show all stages, from the first appearance of fine granules in the cytoplasma of the cells, which stain brilliant orange in Azan preparations, to larger droplets, which finally are extruded and lie as colloid masses among the cells. This cycle can be shown clearly and the identity of those processes in nerve cells with the different stages of secretory activity in gland cells seems sufficiently demonstrated. The physiological meaning of the gland-nerve cells is still unknown and a wide field is opened for future investigation.

Synaptic transmission in the sixth abdominal ganglion of the crayfish. C. Ladd Prosser.

Transmission through the sixth abdominal ganglion of the crayfish was studied by simultaneous recording of impulses entering and leaving the ganglion in response to stimulation of caudal sensory hairs. Flexion of one hair gives rise to one sensory impulse. To set off one efferent unit, however, summation of two to four afferent impulses in different fibers is necessary. No efferent neurone is excited by one incoming impulse. When several efferent units are excited more afferent impulses are required to activate the first efferent unit in a response than to activate later ones. Thus both convergence and overlap play a part in conduction through this ganglion.

Ganglionic delays range from 3 to 30 milliseconds as measured from the time the first sensory impulse enters the ganglion. Most of the fastest units show delays of 5–6 milliseconds, and later ones fall in groups which are multiples of the first. This multimodal distribution of delays is interpreted as indicating the existence of internuncial neurones. A given unit may show fluctuation of 2–3 milliseconds in synaptic delay. The afferent neurones respond to stimuli separated by intervals as short as .01 second, whereas the efferents show recovery times of .05 to .1 second. This synaptic recovery time is longer than that of the fibers, hence no relatively refractory period can be detected in the responses of the individual efferent units.

There are no connections from tactile receptors across the ganglion to contralateral efferent nerves of the sixth segment.

Acetylcholine and eserine have no facilitating action upon the synapse. Eserine is toxic in high concentrations. Nicotine blocks conduction through the synapse. Excess potassium reduces action potentials in the afferent fibers and may block them before affecting the synapse. Adrenaline acts similarly to excess potassium.

It is concluded that those humoral agents which mediate transmission in some mammalian ganglia do not have a similar action in this crayfish ganglion.

July 20

Chemical stimulation of the amphibian ectoderm. L. G. Barth.

Further work on the chemical nature of the amphibian organizer indicates that the formation of the neural plate is due to some general stimulus imparted to the cells by a variety of substances. Earlier experiments using cephalin as an inductor showed that cytolysis occurred in the region of the implant. Following this other cytolytic agents such as digitonin, acids and bases were used and plates of neural cells were induced in the presumptive epidermis. Digitonin in concentrations of .5 to .05 % in powdered egg albumen and buffers at pH 3 and 10 have given positive results by implantation into the blastocoel of *Amblystoma mexicanum.*

With regard to the naturally occurring organizer, it was found impossible to extract it completely with fat solvents. In comparing the action of ether-alcohol extracts with protein residues, the protein residue gave better neural tubes. The ether-alcohol extracts were relatively weak in inducing power. The experiments suggest that the naturally occurring organizer is in the protein residue.

Limb bud transplantation in chick embryos. Viktor Hamburger.

Wing and hind-limb primordia of chick embryos, incubated 2–3 days (25–30 somites) were transplanted to the lateral trunk region or into the coelomic cavity of host embryos of the same stages. In a considerable number of experiments, the anterior-posterior and the dorso-ventral axes were inverted with respect to the axes of the host embryo. The transplants showed complete self-differentiation with respect to form, size and axial pattern.

Transplants located near the spinal cord of the host were supplied by trunk nerves or by nerves branching from the brachial or the lumbo-sacral plexus of the host. The spinal ganglia contributing to the innervation of the transplant reacted always to even small increase in their peripheral fields by hyperplastic growth. In several cases, the number of the motor neurones in the level of the spinal cord which supplied the transplant was likewise found to exceed the number of motor neurones of the normal side, the hyperplasia ranging from 14 per cent to 30 per cent.

Transplants located far ventrally were not innervated by spinal nerves. They showed, nevertheless, normal development and differentiation. The embryonic development of the limbs of the chick is therefore independent of innervation.

Adult organizers and their action in adult tissues. Oscar E. Schotté.

The development of a salamander, Amblystoma punctatum. L. S. Stone (motion picture.)

The development of the common black, yellow spotted salamander, *Amblystoma punctatum,* has been recorded in detail on one motion picture reel showing all the stages from the one-cell egg to the time the larva begins feeding. Rate in development was recorded at various speeds from a few hundred to several hundred times in order to analyse various stages more carefully.

The detailed study in development extends over a period of about four weeks during which time a constant temperature of about 70° Fahrenheit was maintained.

During segmentation one can see clearly the movement of blastomeres, the regional waves of cell division and movement of egg mass due to shifts in the center of gravity. The formation of the blastopore and the development of the neural plate and closure of the neural folds are shown in detail. All the changes in surface development are shown throughout the tail-bud stages from the time at which cilia on the surface of the embryo first come into action to the period when motor activity begins. Occasional local quivering movements in various parts of the embryo are also recorded.

Following these periods in development, dorsal, ventral and lateral views of embryos are shown which carry the growth up to the time the larva begins feeding. The formation of many external features as well as the beginning of peristaltic movements in the intestines are clearly seen.

In appropriate parts of the film are shown clusters of eggs as they are normally laid, circulation in the tail fin, and a view of the larva taking its first meal. The picture begins with a view of a typical habitat and ends with a view of several stages in development from the egg to the adult animal in order to give a comparison of sizes during growth.

JULY 27

The effect of standing on the carbon dioxide content of alveolar air and total ventilation volume. J. K. W. Ferguson and F. A. Hitchcock.

A study of the respiratory exchange during consecutive ten-minute periods of reclining and standing, has revealed in every experiment a decrease of 7–15 per cent in the CO_2 content of alveolar air (confirming Higgins, 1914, Turner, 1927 and Main, 1937). This fall, however, cannot be attributed to overventilation in the usual sense of the word, because: (1) the R.Q. fell during standing in 21 out of 24 experiments, (2) the CO_2 output fell in 12 out of 24 experiments, (3) the total ventilation fell in 9 out of 24 experiments.

When four consecutive periods, with the subject alternately reclining and standing, were studied, it was found that the O_2 consumption and the CO_2 output were greater during the second reclining period than during the preceding standing period. This is interpreted as evidence of oxygen debt and CO_2 retention during standing. It seems probable that this disequilibrium, during standing, between metabolic requirements and the respiratory exchange is due not to inadequate pulmonary ventilation but to inadequate circulation in the dependent parts of the body resulting from the erect posture.

The mechanism of the loss of heat from the human body. Eugene F. DuBois and James D. Hardy.

The three main channels of heat loss are radiation, convection and vaporization. The respiration calorimeter of the Russell Sage Institute of Pathology in New York Hospital measures the vaporization by weighing the moisture that comes from the skin and lungs. It measures radiation plus convection in a stream of cool water that flows through coils in the top of the calorimeter. Radiation is then determined independently by means of a Hardy radiometer pointed in rapid succession at 20 different places on the surface of the body. The total surface is measured and the effective radiating surface calculated as 80 per cent of the total. Convection is then estimated by difference. It has been possible for the first time to separate the factors of radiation and convection in persons exposed to varying atmospheric conditions such as are found indoors.

Two normal men were studied naked at temperatures between 22 and 35°C. Their basal metabolism was uniform throughout this range not rising until a few minutes before the onset of shivering which occurred after exposures of two hours at 22°C. At the higher temperatures there was profuse sweating and practically all of the heat was lost through vaporization. The percentage of heat lost in radiation decreased steadily with rising temperatures. In the neutral zone of 28 to 32°C. convection accounted for 10–15 per cent of the heat loss and radiation 50–60 per cent. Convection was markedly increased by slight movements of the body. With moderate exercise or shivering it accounted for 25–30 per cent of the total loss. An electric fan raised the percentage to 33. Two athletes playing violent squash for 36 minutes showed a 2°C. rise in rectal temperature and about an equal fall in average surface temperature. The total heat lost through radiation increased but little, the percentage fell to 15. Convection during the exercise and recovery period accounted for 5–15 per cent. Vaporization dissipated 70–80 per cent of the heat.

Peripheral inhibition of smooth muscle. Emil Bozler.

The antagonism of vasoconstrictor and vasodilator nerves was studied using perfused frog legs and rabbit's ears and recording the vascular responses by a sensitive flow-meter. Stimulation of the vasodilators of the dorsal roots blocks the action of single volleys of impulses of the vasoconstrictor fibers, whereas repetitive stimulation produces a response. Likewise acetyl choline blocks entirely the action of the first few impulses produced by repetitive stimulation. In an attempt to explain these results a difficulty was encountered in the observation that neither vasodilator stimulation nor acetyl choline antagonise the action of adrenaline. The simplest explanation seems to be the assumption that during inhibition the passage of impulses from vasoconstrictor fibers to the muscle is partially blocked, thereby preventing the formation of the adrenaline-like mediator. It is suggested that the increase of polarisation, which has been observed in other cases of inhibition, is the immediate cause of the partial block. The mediator produced by the vasodilator fibers may be the cause of the change of polarisation of the muscle fibers and, therefore, indirectly also of the partial block produced by the activity of these nerve fibers.

The relationship of tissue chloride to blood chloride. William R. Amberson, Thomas P. Nash, Arthur G. Mulder and Dorothy Binns.

A number of previous investigators have found that amphibian muscles, placed in, or perfused with, isotonic sucrose solutions, rapidly lose their chloride almost completely, while retaining almost all of their potassium and phosphate. When such muscles are soaked in solutions of varying chloride content, the tissue chloride varies directly with that of the external fluid. Such observations have led to the conclusion that muscle chloride is extra-cellular, a view reinforced by its low concentration in this tissue.

Other students have recently attempted to extend this concept to other tissues, and to the mammalian body. In studying this literature it occurred to us that it would be useful to know whether the chloride of the mammalian tissues can be diminished when the plasma chloride is lowered. We have found it possible to produce very radical diminutions in the plasma chloride by a modification of the method of total plasmapheresis described by Stanbury, Warweg, and Amberson (*Am. Jour. Physiol.,* 1936, 117, 230). We make up our artificial plasma with sulfates instead of chlorides, adding chloride-free gum acacia, and suspending in this plasma ox red blood corpuscles which have been rendered chloride-free by many washings through sulfate-Ringer-Locke solution.

By long perfusion of the mammalian body we are able to remove most of the chloride before death ensues. In some tissues, such as skeletal muscle, liver and kidney, the tissue chloride falls off in direct proportion to the plasma chloride, as perfusion proceeds. In other tissues the straight line through all the points does not pass through the origin, but shows a y-intercept, suggesting that a portion of the chloride is indiffusible, and presumably intra-cellular. The stomach and spleen have particularly large intercepts of this character.

The central nervous system resists removal of chloride, whereas most of the chloride of peripheral nerve may be washed away. The brain and cord chloride is held either (1) because it is largely intra-cellular, or (2) because the sulfate ion is unable to penetrate into the brain tissue.

Certain tissues, such as tendon and lung, have chloride concentrations so high that it is impossible to explain it all by allotting it to extracellular fluid.

AUGUST 2

The use of diatoms from geological excavations at Clovis, New Mexico, as indicators of water conditions. Ruth Patrick.

Mammoth Pit lies between Clovis and Portales in the Staked Plains region of New Mexico. The stratigraphy is as follows: The lowest stratum is coarse gravel devoid of diatoms. Above this is a stratum of speckled sand, in which is a diatom flora which seems to indicate a fresh to brackish water condition by the dominance of *Anomoeoneis sphaerophora* (Kütz.) Pfitzner, *Amphora ovalis* Kütz., and *Amphora ovalis var. pediculus* Kütz. Horse skeletons are found in this level. The next stratum is a "bluish clay" of about the same constituents as the speckled sand plus considerable carbonized vegetable matter. In the lower part of this stratum, a very rich diatom flora consisting mainly of fresh water species such as *Eunotia arcus* Ehr., *Cymbella affinis* Kütz. *Fragilaria brevistriata* Grun. and *Synedia ulna* (Nitzsch.) Ehr. was laid down. The change in abundance and also in the kind of species from the previous level points to a freshening of the water. This agrees with other geological evidence that this was a period of much higher precipitation than now exists in New Mexico. Mammoth skeletons are most abundant in this level of the stratum. Passing from bottom to top of this stratum the typical fresh water species disappear. The dominant species near the top are the brackish or alkaline water types such as *Epithemia argus* Kütz., *Rhopalodia gibba* (Ehr.) Müll., and *R. gibberula* (Ehr.) Müll. Coincident with the change in diatom species, the mammoth bones disappear and bison skeletons become much more numerous. The top stratum consists of brown dune sand devoid of diatoms. It is in the bottom of this layer that the bison bones disappear. Thus the change in water conditions as indicated by the diatoms seems to coincide with a change in the fauna as shown in this stratigraphy.

Gemmipary in Kalanchoe rotundifolia and other Crassulaceae. Harry N. Stoudt.

The phenomenon of vegetative propagation in Crassulaceae has received much attention from students or morphology and physiology. A comparison of the morphological development of plantlets of this species with other members of the family should aid in understanding more adequately the phenomenon so characteristic of the group.

Yarbrough (1936) reports that apparently mature parenchyma cells in the base of the petiole of *Sedum Stahlii* resume mitotic activity to form plantlets when the parent leaf is removed from the plant.

Stoudt (1934) published an account of vegetative propagation in *Byrnesia Weinbergii*. In this species a plantlet forms at the base of the sessile leaf from a residual meristem that is undifferentiated into plantlet rudiments.

Freeland (1933) discusses this phenomenon in *Bryophyllum crenatum* in which plantlets develop from residual meristems in the notches of the parent leaf. He finds that the amount of differentiation of plantlet rudiments varies. A stem primordium only may be formed by the time the parent leaf is mature.

A nine-millimeter leaf of *Kalanchoe rotundifolia* reveals a meristematic region on the adaxial surface of the petiole. This region is undifferentiated into plantlet rudiments but when the leaf attains its maximum size, three to four centimeters in length, leaf and stem primordia have formed. The bud then remains dormant until the leaf is severed from the parent plant.

Root, stem and leaf primordia are usually visible in the notches of the mature leaves of *Bryophyllum calycinum* according to Naylor (1932) etc. while in *Kalanchoe daigremontiana* and *K. tubiflora* plantlets consisting of root, stem, and leaf primordia are visible macroscopically even before the parent leaf has attained maximum size.

Thus there is a definite sequence into which the various species arrange themselves in respect to the degree to which the meristematic cushion becomes differentiated by the time the parent leaf is mature. Their greatest differences are in the stage of development attained by the meristem, or organ rudiments derived from it, at this time.

Pollen analysis of the air in relation to hay-fever. A. O. Dahl.

It is essential for the successful diagnosis and treatment of hay fever ("pollenosis") that detailed data concerning the concentration of pollen in the air of any species at a given time or locality be made available. Records of the pollen content of the air have been obtained by exposing each day an oil-coated slide out of doors for 24 hours. The pollen grams observed in 25 systematically distributed low-power fields are identified and counted. The approximate number of pollen grains per cubic yard of air can be calculated by use of physical formulae. The pollen grains involved in hay fever in Minnesota vary from approximately 15 to 80 micra in diameter.

Hay fever is a regional problem and atmospheric data from one locality will not apply in a detailed manner to another place. In Minnesota, pollen concentrations of clinical interest occur between late March and early November. For purposes of clinical convenience, the pollens found in the air during the entire season have been placed into 16 groups. Thus, in diagnosis, one scratch-test for each group will test the patient's sensitivity to all pollens present during the entire season. Prefaced by such procedure, successful therapeutic measures can be instituted. (The complete report is to be published in joint authorship with Dr. C. O. Rosendahl and Dr. R. V. Ellis, under whose direction the study has been carried on for the last 5 years at the University of Minnesota).

AUGUST 3

A convenient test of physical agents as producers of dominant lethals. P. W. Whiting.

Dominant lethals occurring in the spermatozoa cause failure of development of zygotes. Since in bisexual reproduction eggs also fail to develop unless fertilized, the two types of male sterility, due to (1) lack of sperm and (2) dominant lethals, cannot be statistically distinguished. In *Habrobracon,* reproducing males by haploid parthenogenesis, matings with males lacking sperm result in as many progeny per day (♂♂) as cultures from unmated females (4.73 ♂♂) or as matings with untreated males (1.21 ♂♂, 3.30 ♀♀). If fathers are X-rayed, males per day are not increased while females per day are decreased (to 2.22 with 2,500 R, 0.62 with 5,000 R, 0.16 with 7,500 R, 0.07 with 10,000 R, and 0 with 20,000, 40,000 or 75,000 R). Since neutrons induce dominant lethals and ultra-short (one-meter) radio waves do not, only the former may be expected to induce recessive lethals and visibles.

Cytological observations on colchicine. Bernard R. Nebel.

The action of the alkaloid colchicine on mitosis was studied in the following material:

Stamen hairs of *Tradescantia,* roots and shoots of *Zea, Vicia, Tomato, Tagetes, Antirrhinum, Trifolium, Papaver, Dianthus, Solanum,* and *Lilium,*—testes of *Podisma,* eggs of *Asterias* and *Arbacia.*

In *Tradescantia* all stages of mitosis are easily seen in life. The cells will continue to divide in salt sugar solutions to which drugs may be added.

Colchicine in concentrations of 5×10^{-4} to 5×10^{-5} molar will stop mitosis during metaphase. In concentrations of 5×10^{-5} to 10^{-6} molar it tends to produce binucleate cells. When blocked in metaphase the plate in *Tradescantia* is characteristically tilted, since a true spindle is not developed. A comparative study of phenyl-, amyl-, propyl-, ethyl urethane and chloral-hydrate showed that these anesthetics within their respective reversible concentration ranges will only occasionally produce binucleate cells. There is no particular evidence of a metaphase block with the urethanes. In studying the action of colchicine on the developing egg of *Arbacia punctulata* it was necessary to use fixed material to determine the nuclear stage accurately. Colchicine applied 10 minutes after insemination will block the first cleavage metaphase in concentrations above 10^{-4} molar. Between 6.5×10^{-5} and 3.5×10^{-5} molar nuclear divisions of abnormal type proceed while cleavage is impeded. The rhythm of nuclear division may persist so that when controls are in third cleavage metaphase the eggs to which colchicine was added 10 minutes after fertilization will also show approximately 4 groups of chromatin, but the plates are hypoploid, often containing only one to six separate chromosomes, which may partly represent fused units. The micronuclei which form are not far apart from one another and their resting stage is relatively short; with lower concentrations the rhythm of division is not affected in the early cleavages, but the subsequent development of the larvae is markedly stunted even at 10^{-6} molar. Colchicine in increasing concentrations thus first interferes with the normal course of cleavage, then astral rays are prevented from forming, next the spindle is reduced in size and finally obliterated. Meanwhile chromosomes become pycnotic during a prolonged metaphase; they fail to divide orderly; the abnormal division may separate entire chromosomes rather than split halves.

In the plants, in which root and shoot growth was studied (Ruttle) cuttings and seedlings respectively were subjected to colchicine treatments by immersion in aqueous solutions primarily. The active concentration ranges were found to be the same as in *Arbacia* and *Tradescantia.* All genera showed marked reactions, the tomatoes being the least sensitive. Where plant meristems were treated, the resulting tissues showed markedly irregular growth—incised and crumpled leaves as well as chlorophyll defects. Cytological investigation showed necrotic cell lineages and multinucleate cells in varying degrees. The drug is being studied further as an agent which may induce mutations and polyploidy.

In coöperation with other investigators, some physiological effects of colchicine were investigated. Respiration was tested on *Arbacia* eggs with colchicine 1.8×10^{-5} to 7.5×10^{-5}. Respiration did not vary from the control in any of these concentrations (Tyler).

Pectinmethoxylase (pectase) from tomato juice showed inhibition which is of doubtful significance since with higher concentrations of colchicine a precipitation was observed in the reaction mixture (Kertesz).

The nitroprussiate reaction for $S-H \rightleftharpoons S-S$ groups gives a color reaction in stamen hair cells of *Tradescantia,* the color being located in the chromonemata and in certain plasmatic granules. Cells under colchicine gave the same reaction (Medes).

No significant influence was observed on the action of carbonic anhydrase from blood.

No inhibition occurred of the reduction of methylene-blue by yeast or blood with and without admission of air.

No sensitization of *Arbacia* eggs to X-rays was obtained by the addition of colchicine, when nuclear abnormalities were used as a criterion.

Demonstration of vital staining preserved in paraffin sections of lamprey embryos (Bismark Brown method). R. Weissenberg.

In 1929 I recommended Bismark-brown for localized vital staining of the egg of lamprey because it is very easy to preserve these stained areas for paraffin sections.

The quick-working method which I am using now in my studies of localization on lamprey egg is a very simple one. It is based on the surprising fact that Bismark-brown as a vital dye is alcohol-proof without further treatment in contrast to Nile-blue sulfate.

I fix the embryos in a mixture of 1.5 parts of absolute alcohol and 0.5 part of acetic acid for ten minutes, wash in absolute alcohol for a few minutes, and transfer them directly into cedar oil.

The stained areas are very well preserved by this simple method and the eggs can remain in the cedar oil for years without any loss of the dye. They can be studied in the cleared condition in the cedar oil as total preparations, or they can be imbedded in paraffin at any time and cut with excellent preservation of the vital stain within the sections.

The slides demonstrated are balsam preparations seven years old and still give a true representation of the stained areas of the living organism. The preservation obtained by this method is complete also in the finer localization of the staining. Carriers of the vital staining in the egg of lamprey are chiefly the yolk granules because here, in contrast to most amphibian eggs, pigment granules are missing in the earlier stages of the embryo.

Microfilm on some experiments on isolated amphibian germinal vesicles. William R. Duryee.

The film shows colloidal changes in the frog ovocyte nucleoplasm, nucleoli, and chromosomes brought about by relatively slight changes in the Na, K, Ca chloride concentrations of the medium. H^+ ions reverse the normal negative charge on the nuclear components to positive. When this change is gradual enough, as with 0.003 N HCl, a dark converging " ring " forms from flocculating particles in the approximate pH region of 4.0 to 5.0.

Ca, Mg, Cu, Hg and basic dyes behave similarly to H^+ ions in causing a phase separation and an appearance of chromosomes from a previously transparent nucleus. On the other hand, K and Na and especially OH^- tend to disperse the nuclear colloids, thus stretching and separating the chromosome pairs, and at the same time making the nucleus transparent. Within narrow limits these changes are reversible.

In *Triturus pyrrhogaster,* the "Binnenkorper," or first maturation spindle anlagen situated at the center of the germinal vesicle, can be made to swell and separate the chromosomes radially, but not in typical bipolar directions. *Rana fusca* appears unique in having a differentiated coagulable capsule around the chromosomes, which may be important in forming the denser portions of the spindle. In *R. pipiens* the contraction of this material under the influence of calcium is less striking.

Similar changes, including phase separation and violent contraction of the chromosomes, occur when acid fixatives are added or when the nuclei are exposed to their disintegrating cytoplasm. Hence this latter effect may be termed *autofixation.* Prominent differentiated areas or sac-like projections of the nuclear membrane reversibly swell and shrink in bases and acids respectively. Such structures are obliterated by fixatives. It is concluded that in the forms studied Darlington's assertion as to the absence of a nuclear membrane must be modified. With merely fine forceps and pipette it is easily possible to isolate various components of these giant nuclei (0.8 mm. diameter),— e.g. nuclear membrane, spindle anlagen, nucleoli, and chromosome pairs (100–200μ)—during the fall, winter and early spring months of the year.

Cortical cytolysis of the echinoderm egg. Robert Chambers. (Motion picture.)

Motion pictures were taken of those experiments designed to demonstrate the physical properties of the cellular cortex particularly in marine ova. Nearly all experimentally induced effects on living cells are extremely transitory. A later study of the motion picture record permits of a more careful and complete analysis of these phenomena. For example, the film may be slowed down, stopped at any point or reversed at will. The film presented at the seminar is a compilation of many of these experiments.

Included in the film is a scene showing the spontaneous coalescence of a denuded *Arbacia* egg with an oil drop. From such a record it is possible to measure with considerable precision the diameter of the oil drop at the instant of coalescence. These data are essential if the coalescence phenomena are to be treated quantitatively, yet it is practically impossible to obtain the oil drop sizes in any other way.

Another scene shows the natural elevation of the cellular surface and the reaction of this surface to mechanical injury. Over-insemination of an immature *Arbacia* egg results in the formation of many large insemination cones. Gentle manipulation with a microneedle will cause these cones to run together, thus bringing about an elevation of a continuous film which is separated from the granular cytoplasm by a liquid space. If this elevated film is ruptured at one spot with a microneedle, the entire film will rapidly disintegrate. The underlying cytoplasm becomes exposed and it quickly becomes converted into an irreversible coagulum.

The following phenomena, *inter alia,* are also demonstrated: cytolysis of starfish eggs in hypotonic sea water, effects of tearing eggs in pure salt solutions isotonic with sea water and the shrinking of the cortex of one blastomere following the puncture of the other.

The photography was done by Mr. C. G. Grand.

August 10

Some aspects of normal and regulative development in the colonial ciliate, Zoöthamnium alternans. F. M. Summers.

A remarkable number of studies on metazoan "organizers" have already demonstrated the importance of extrinsic factors for determination in specific parts. It was felt that additional information about these factors could be gained by applying operative techniques to an animal type in which, presumably, the relationships of parts have not attained so great a degree of complexity. *Zoöthamnium alternans* is a protozoan colony whose cells collectively possess in some degree many of the attributes of an individual organism. It is admirably adapted to this type of work for many reasons, particularly by virtue of the precision with which the characteristic colonial pattern develops.

One of the most important consequences of this study of more than 200 normal and operated colonies is the demonstration of qualitatively different physiological relations between cells at different locations on the colonial framework. Under normal conditions a specific pattern unfolds. When the cell at the apex of the frond-like colony is cut away some cell of a lower order, one whose complete developmental potentialities are never otherwise expressed, assumes the dominant generative functions and the normal colonial pattern perseveres in the parts regenerated by it. These results are intelligible in terms of what Child (1929) calls physiological correlation: the relations of dominance and subordination between parts. Apical control appears to be continuous and quantitative.

In this organism the transformation of the apical cell into an ex-conjugant initiates a developmental phase which furnishes another clue to the general nature of apical control. About four days after the union of gamonts, even before ex-conjugant generations are produced, the normal developmental relations are upset in an unusual way. The first three or four branches below the conjugant level begin to develop out of all proportion to the normal expectations. Each branch develops almost as an individual colony. Its cells divide precociously, forming secondary and even tertiary branch strains. The greatest effect obtains on the branch nearest the conjugant and diminishes basally as a gradient; the basal branches are apparently unaffected.

Under varying physiological conditions in the apical cell the coördinating influences exerted upon the mitotic activity of neighboring cells may be inhibitory (as shown by the regulative response after the apical cell is removed) or excitatory (when the apical cell is transformed into an ex-conjugant). The precocious development does not occur when the apical cell is present *or* when it is dissected away; it appears to be effected by some new quality in the coördinating mechanism arising in consequence of conjugation activities in one particular cell—the apical cell. These results invite the conclusion that the integrative factors in a colony of *Zoothamnium* are qualitative and discontinuous.

Morphology, behavior and reproduction in Type A and Type B of Chaos chaos Linnaeus, the giant multi-nucleate amoeba of Roesel. A. A. Schaeffer.

Chaos chaos Linnaeus 1767, the first amoeba to be discovered, was described by Roesel von Rosenhof, a painter of miniatures, in 1755, in Germany. It has been seen 5 times since then: in 1900 by H. V. Wilson, North Carolina; in 1902 by E. Penard, Switzerland; in 1916 by W. A. Kepner in Virginia and by A. A. Schaeffer in Tennessee; in 1936 by A. A. Schaeffer in New Jersey.

The general morphology and behavior of this amoeba are so much like those of the common laboratory amoeba, *Chaos diffluens* Müller, 1786, that there is strong probability that both amoebas belong to one and the same species. Conclusive evidence of such relationship is, however, still lacking and therefore, until such evidence is found, the two taxonomically specific names, *C. chaos* and *C. diffluens,* will be used to avoid confusion.

C. chaos falls into at least two types which are distinct in some morphological details and also in antigenic reactions. Type A has discoid nuclei and divides usually while freely rolling around on the substrate. Type B has broadly ellipsoid nuclei, and divides while fastened to the substrate. The nuclei of type A are larger than those of type B. With the collaboration of Dr. J. A. Harrison, preliminary antigenic tests were made in which it was found that there is a marked difference between the two types, type B standing closer to *diffluens* than to type A.

Three of the most striking differences between *chaos* and *diffluens* are: in size, *chaos* being from 50 to 500 times as large as *diffluens;* in number of nuclei, *chaos* being multinucleate (up to 1,000 or more); in reproduction, *chaos* dividing at any single division, into 2, 3, 4, 5 or 6 daughters, with a strikingly marked mode at 3 daughters.

Pieces cut from *chaos* grow up to full size, whether the piece contains one or more nuclei.

Both types give off, when crushed, a strong cucumber-like odor which can readily be detected when only one amoeba is crushed on a slide. In *diffluens* this odor is also present but to a much smaller degree.

Observations upon the chemical composition and the metabolism of a larval parasitic nematode. Theodor von Brand.

The experiments were performed with an immature *Eustrongylides* from *Fundulus heteroclitus.* The red color of the worms is due to the presence of hemoglobin in the body fluid. With its low fat and high glycogen content the general chemical composition resembles that of the adult *Ascaris.* The worms consume per unit weight much less glycogen than *Ascaris,* if kept in saline at 37° C. under aerobic conditions. They are able to keep their glycogen level high, even if their hosts starve for a long time and lose during this starvation period more than half of their polysaccharide stores.

Observations and experiments on sex change in the adult American oyster, Ostrea virginica. Paul S. Galtsoff.

Sex of oysters was determined by inducing ovulation or ejaculation by increased temperature and chemical stimulation. During the summer of 1936 individual spawning records of 203 adult oysters were obtained and the discharged products microscopically examined. Oysters were measured, carefully marked by numbers engraved on the shell of each, and transferred to Milford, Connecticut, to be kept in tidal tanks through the winter. The same procedure was repeated during the summer of 1937.

Results showed that 9.7 per cent of the oysters reversed their sex; the percentage of reversals being higher among females (13.1 per cent) than among males (8.0 per cent). Mortality during the year was only 7.04 per cent and failures to react, due to lack of gonad development, was only 1.07 per cent, indicating healthy conditions under which oysters were kept.

In the sex-reversed males the physiological set-up of the organism changes with the sex; typical female reaction, characterized by the rhythmicity of contractions of adductor and maintenance of a constant tonus level, develops in the former male. In several instances development of this reaction lagged and the newly-formed female retained physiological characteristics of the male, i.e., eggs were discharged through the cloaca instead of through the gills and rhythmical contractions of adductor were absent. All sex-reversed females reacted as true males.

It is concluded that female reaction developed as a secondary adaptation providing mechanism for dispersing eggs throughout the water. From the simultaneous occurrence of sex reversal in the oyster population the conclusion is made that Orton's theory of metabolism change (protein to carbohydrate) as the sex-determining factor in *O. edulis* is not applicable to *O. virginica.*

August 17

A sea water buffer for marine eggs. Albert Tyler and Norman H. Horowitz. (This paper has already appeared in full in *Science,* July 23, vol. 86, pp. 85–86.)

The effect of CO_2 upon the oxygen capacity of the blood of some fresh-water fish. Edgar C. Black and Laurence Irving.

Conditions of respiration for fish differ from the respiratory conditions for mammals. The respiration of fresh-water fish must proceed in a medium in which the pressure of oxygen is always less and the pressure of carbon dioxide usually greater than in atmospheric air. At different levels in the water, pressures of gases are altered by the temperature changes. Types of blood which are suitable for the transport of CO_2 and oxygen under one set of conditions might be quite unsuitable under another set. The characteristics of the blood of the carp (*Cyprinus carpio L.*) and the common sucker (*Catastomus commersonii*) show examples of two types of blood, each suited for a different and limited range of pressures of oxygen and CO_2.

Oxygen dissociation curves obtained from those two species are not as sigmoid as are those for mammalian blood. The presence of 5 or more mm. CO_2 (for the carp 10 or more) prevents the complete saturation of whole blood, even at high partial pressures of oxygen. This effect of CO_2 is quite different from the familiar effect of CO_2 upon mammalian blood. In the presence of relatively high pressures of CO_2 the blood of the carp is suitable for the transport of small quantities of oxygen, while the blood of the sucker would be quite useless.

At very low pressures of CO_2 and high pressures of oxygen in the water the blood of the sucker can serve to transport much more oxygen than that of the carp.

Hemolysis by the addition of saponin to the blood of the sucker, carp and bowfin (*Amia calva*) abolished the effect of CO_2 upon the oxygen capacity at high pressures of oxygen (150 mm.). The CO_2 effect is in part at least dependent upon the integrity of the corpuscles.

Oxidative mechanisms in the resting and fertilized sea-urchin egg. Irvin M. Korr.

Since cyanide inhibits certain iron-containing systems, and since pyocyanine, a bacterial respiratory pigment, functions as a "hydrogen-carrier," it was possible to vary the relative proportions of respiration going through simple, non-ferrous carrier and that going through the cytochrome-indophenol oxidase system; by adding KCN and pyocyanine, separately and in various combinations, to sea urchin eggs. The respiratory rates of the untreated fertilized and unfertilized sea urchin eggs, and those in which the mechanisms had been altered as above, were measured at different temperatures. These experiments were designd (1) to give the temperature relations of the two types of respiration, (2) to throw further light upon the factors determining temperature coefficients of cellular respiration and (3) upon the change in oxidative rate and mechanism that occurs upon fertilization of the sea urchin egg.

It was found, first (in partial confirmation of Rubenstein and Gerard, 1934), that the temperature coefficient of unfertilized eggs was much higher than that of the fertilized egg. The effectiveness of KCN was found to increase with temperature.

Increasing the respiration of the fertilized egg with added hydrogen-carrier did not appreciably change its temperature coefficient. Fertilized eggs, in which the iron system had been maximally inhibited and the respiration restored to or above normal with pyocyanine, also had the same temperature coefficients as the untreated fertilized eggs.

In the unfertilized egg, whose respiration is cyanide-stable, the addition of carrier not only increases the rate of respiration, but also, above a certain concentration, lowers the temperature coefficient. A concentration of pyocyanine which increases the respiratory rate to that of the fertilized egg also lowers the coefficient to that of the fertilized egg.

The results, in conjunction with older work, lead to the conclusions that (1) respiration through a simple non-ferrous carrier and that through the cytochrome system do not, per se, have different temperature coefficients, that (2) these H-transfer mechanisms are the rate-controlling link in the respiration of the fertilized and unfertilized egg. (3) Temperature coefficients are largely determined by the ratio of the rate at which H-atoms are transferred, from substrate to oxygen, to the maximum rate at which they can be produced by the substrate-dehydrogenase systems. The more nearly the H-transfer rate approaches the maximum H-production rate, the lower the temperature coefficient, and vice versa—within the limits set by the fertilized and unfertilized egg.

Methods for the study of rapid chemical reactions and their application to the kinetics of enzyme-substrate and enzyme-inhibitor compound formation. Kurt G. Stern and Delafield DuBois.

The observation of spectroscopically defined enzyme-substrate and enzyme-inhibitor compounds, made in the course of recent studies on catalase and peroxidase, offers an experimental approach to the detailed analysis of the mechanism of action of these enzymes. A photoelectric method for the recording of such processes and some preliminary results were reported last year (K. G. Stern and D. DuBois, *J. Biol. Chem., 116,* 575 (1936)). This method has since then been improved by replacing the single photoelectric cell by a differential photometer containing two photocells. The technique of mixing by injecting one reactant into the solution of the other with a spring gun has been retained. The new apparatus is quite insensitive to any but color changes. In addition, a simple spectrographic method has been developed which permits the recording of fast reactions without the use of photocells or electric instruments. This is achieved by replacing the plate holder of a spectrograph by a falling plate camera. While the plate is falling, the trigger of the spring gun is released and mixing is complete within 8 to 27 milliseconds. The time is recorded by a rotating time marker or a Neon tube flash circuit. With this method the reaction of catalase and methemoglobin with hydrogen peroxide, ethyl hydrogen peroxide, hydrocyanic acid, and hydrofluoric acid has been studied. The reaction rate is greatly dependent on the concentration of the reactants and on their ratio, as would be expected from a bimolecular process. The reactions studied appear to be slower than the reactions of hemoglobin or hemocyanin with oxygen or carbon monoxide which have been measured by Hartridge, Roughton, and G. A. Millikan with the flow method. Only when a considerable excess of substrate or inhibitor over the catalysts is employed does the rate of the reactions studied by the present authors approach the length of the mixing time which is, of course, the limiting factor in such experiments. Inasmuch as the rate of fall of the photographic plate may be varied within wide limits (0.3 to 29 cm. per second), a wide range of reaction rates may be studied. The use of supersensitive panchromatic plates permits the recording of changes of light absorption in the red and green region of the spectrum at rates of fall of the plate corresponding to an exposure time of less than 0.002 second for an individual spectrum. The continuous strip of spectra recorded on the plates corresponds to 350 individual spectra.

August 24

Mechanism of cellular death by freezing. B. Luyet. (The essentials of the paper were published in the August issue of *Biodynamica.*)

Binding and penetration of bivalent cations in Elodea cells. Daniel Mazia.

The fact that *Elodea* cells contain soluble oxalates in their vacuoles makes it possible to study by a direct method the binding of Ca, Sr, and Ba ions. By subjecting the cells to strong electric currents or to ultraviolet radiation or to plasmolysis and deplasmolysis, one can set free the bound Ca in their protoplasm, which can then be observed as a precipitate of distinct calcium oxalate crystals in the vacuole. The Ca must come from the protoplasm, for the cells are kept immersed in distilled water or non-electrolyte solution.

That the Ca actually is bound in living cells is indicated by the fact that it cannot be washed out by prolonged immersion (up to 14 days) in distilled water, although it can easily be removed by a few minutes of washing in a citrate solution and replaced then by a few seconds of immersion in a 0.01 M $CaCl_2$ solution. Further studies on leaves from which the Ca has been removed by citrate show that it can be rebound to a maximum level from Ca solutions as dilute as 5×10^{-6} M, the time required increasing with the dilution. This binding is influenced by Na and also by K ions, so that when the Na/Ca ratio is greater than about 100, the binding of Ca is largely prevented.

It is possible to substitute Sr and Ba in the place of Ca in the protoplasm. They are bound in the same way as Ca. Cells in which they have been substituted for Ca seem to function normally, but when subjected to agents regularly causing the release of Ca show characteristic crystals of strontium oxalate or barium oxalate in their vacuoles.

The Sr ion penetrates the protoplasmic layer easily, even when there is no concentration difference between the Sr inside and outside or even a higher concentration inside. It can be demonstrated that this transport requires first the binding of the Sr in the protoplasm. If Na or K is added to an Sr solution in sufficient concentration to prevent the binding of Sr in the protoplasm, the penetration of Sr does not occur, whereas, in the control, a pure $SrCl_2$ solution of the same concentration, the transport does occur, and crystals of strontium oxalate can be seen in the vacuole.

Factors governing cellular responses to nitro and halo phenols. G. H. A. Clowes, A. K. Keltch and M. E. Krahl.

A recent comparison of respiratory stimulating and cell division blocking effects of three closely related compounds having pK values ranging from 4.1 to 4.5, mononitrocarvacrol, dinitrocarvacrol and dinitrothymol, shows that the first and third substances exert little or no effect on respiration but block division at the extraordinary dilution of 2×10^{-6} M. The second increases respiration almost four-fold to a peak at 10^{-5} M, at which concentration division is blocked. A fourth structurally related substance, 2,4-dinitro-*o*-isopropyl phenol, having a pK of 3.0, produces a moderate effect, giving a respiratory peak and cell division block at 10^{-3} M. These results lend further support to the conclusion previously reached that, while the substituted phenol anion undoubtedly exerts a significant effect inside the cell, it is quite impossible, from a knowledge of only the dissociation constants of such substituted phenols, to predict with certainty the range of concentrations, if any, in which a given compound will affect either cell respiration or division or both.

From experiments conducted in 1935 and confirmed in 1936, in which varying numbers of eggs were used in a constant volume of sea water medium, certain substituted phenols were observed, contrary to general experience with anesthetics, to block division at greater dilution when larger numbers of eggs were employed and vice versa. This was believed to be attributable to a rise in intracellular acidity due to CO_2. At last year's meeting it was demonstrated that with varying CO_2 tensions, incapable in themselves of blocking division, the division-blocking effect of substituted phenols was greatly enhanced.

In an attempt to evaluate the relative rôle of undissociated substituted phenol molecule and anion, the dissociation constants of some thirty substituted phenols have been determined during the past winter and used in analyzing the respiratory stimulating and cell division blocking effects obtained with the compounds in question at fixed exterior and varying interior acidities. These will be reported in the following paper.

On the assumption that the substituted phenols penetrate the living cell only as undissociated molecules, for a given total concentration of the phenol, the intracellular concentration of phenol molecule and phenol anion in an intracellular aqueous phase may be calculated for any levels of extracellular and intracellular acidity.

The possible rôle of acidic dissociation in the physiological effects produced by nitro and halo phenols. M. E. Krahl, G. H. A. Clowes and A. K. Keltch.

From experiments performed during the summer of 1935, using a constant extracellular pH of 7.5 and a constant intracellular pH approximating the normal

6.8, the intracellular concentrations of phenol molecule and phenol anion necessary to give 50 per cent reversible inhibition of division and approximately optimum respiratory effect (where this effect was present) were calculated for 30 substituted phenols. The necessary concentration of phenol molecule was found to vary from 3×10^{-10} M for 2,4-dinitro-*o*-cyclohexyl phenol to 3×10^{-4} M for *m*-nitrophenol. The necessary concentration of phenol anions likewise varied over a wide range, from 1.0×10^{-7} M for 2,4-dinitro-*o*-cyclohexyl phenol to 2×10^{-4} M for 2,4-dinitro-*o*-isopropyl phenol. In confirmation of our previous work and contrary to the results of Tyler and Horowitz (*Proc. Nat. Acad. Sci.*, 23: 369, 1937), picric acid and *o*-nitrophenol, when free of impurities, produce no reversible stimulation of respiration or reversible cell division block. It is clear that no final conclusion regarding the precise rôles played by the phenol anion and undissociated phenol molecule can be reached from such experiments, and that there is some as yet undetermined factor involved. The conclusions of Tyler and Horowitz, who used a limited number of compounds which happened to fall, for the most part, in the middle of the above series, are therefore not justified by the evidence at present available.

With a constant extracellular pH and a decreasing intracellular pH, the ratio of intracellular anion to intracellular substituted phenol molecule decreases, while the concentration of phenol molecule is independent of intracellular pH and dependent only on the total extracellular concentration of phenol and the extracellular pH. In experiments on fertilized eggs of *Arbacia punctulata,* it was found, as anticipated, that at constant extracellular pH of 6.7, the concentrations of 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol, 2,4,5-trichlorophenol, 2,4-dichlorophenol, and *m*-nitrophenol necessary for 50 per cent division block were not affected by decrease in the intracellular pH, while the optimum levels of respiratory stimulation were decreased with decreasing intracellular pH, this decrease in respiratory optimum being largest with 2,4-dinitrophenol and 4,6-dinitro-*o*-cresol which have pK values of 4.1 and 4.4 respectively, and small or negligible with 2,4-dichlorophenol and *m*-nitrophenol which have pK values of 7.7 and 8.3 respectively.

Depolarization of muscle and nerve membranes by organic substances. Rudolf Höber and Bernard R. Nebel.

It is fairly generally accepted that some surface film of muscle and nerve fibers is the seat of a polarized state resulting from the high content of the interior of the fibers in free K ions and from the selective permeability of this film to cations. Furthermore, it is believed that the negative electric wave sweeping along the fibers after excitation is indicative of a local and reversible propagated depolarization due to an increase of ion permeability. This increase would be the result of an electro-chemical reaction, which involves a structural alteration of the surface film. Since the excitation process has been shown to be connected with an increased metabolic activity of the fibers, it seemed worthwhile to study whether organic compounds either identical or more or less related to normal constituents of the fibers would bring about depolarization.

Experiments were performed on sartorius muscles and sciatic nerves of the frog, complemented in coöperation with Dr. M. Andersh, by studying nerves of the spider crab. Injury potentials were measured, following the usual procedure.

The experimental result is this, that not only certain organic cations, comparable to the normally penetrating K ions, but also certain organic anions and non-electrolytes are enabled to depolarize the surface membrane, as disclosed by the arising electronegativity. The active cations concerned are those of higher dialkylamines and of alkaloids, the anions those of higher fatty acids and bile acids, the non-electrolytes anesthetics and saponin-like compounds. All these substances are likewise significant by their cytolytic power, which is associated with a polar structure of their molecule, with surface activity and lipoid-solubility. Lytic effects frequently appear to be irreversible. But under certain conditions, e.g., with the fatty acids by shifting the pH from a more acid to a more alkaline reaction, the depolarized state can be returned to the normal polarization.

These statements tempt one to raise the question whether reversible cytolysis may play a rôle in producing the traveling negativity, the propagated reversible disturbance of the surface film of the excited fibers. Support may be lent to such an assumption by the facts that the phosphatides, characteristic constituents of the plasma membranes, are containing surface-active higher fatty acids and that electric currents, in passing artificial membranes, comparable to the action currents accompanying excitation, have been demonstrated to alter the ion concentrations, particularly the H ion concentrations in the electrolyte solutions bordering the membranes. Under such conditions, in the membranes various physico-chemical or chemical events could be released.

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On some conditions determining sub-cooling in plant tissues. B. J. Luyet and E. L. Hodapp.

Organisms found in frozen water or on the frozen ground are said to be sometimes killed by a slight concussion incapable otherwise of harming them. This would be attributable to the sudden freezing of the sub-cooled tissues under the action of the concussion. In the present experiments we studied the conditions determining sub-cooling and freezing after sub-cooling, in the potato tuber. Sub-cooling was found to occur with about the same frequency in living and in dead tissue. The temperature to which the material was heated before being cooled had an evident influence on preventing sub-cooling. Freezing of the sub-cooled tissues can definitely be induced by concussion, although the relatively high percentage of inefficient shocks indicates that some unknown factors, uninfluenced by concussion, hold the system in the sub-cooled condition.

On the double freezing point of some living tissues. B. J. Luyet and Sister P. M. Gehenio.

Some plant tissues have been found to present sometimes two freezing points, one a few tenths of a degree below zero and the other about a degree lower. In the present work we investigated the conditions in which one obtains the double freezing-point in the potato tuber. From a large number of determinations it results that it is a character of living tissue and that a congelation of the material at the first freezing-point does not kill it, while at the second it does. The presence of the double plateau in the freezing curve, and its shape, have been studied in terms of the cooling velocity, of the occurrence of sub-cooling, of the size of the piece of tissue, of its degree of desiccation or of imbibition, and of the type of thermometric device used. The possible factors responsible for the double freezing point are discussed.

Transverse electric impedance of the squid giant axon. H. J. Curtis and K. S. Cole.

The transverse electric impedance of the giant axon of the lateral mantle nerve of the squid has been measured by means of a Wheatstone bridge over a frequency range from 200 to 2,500,000 cycles per second. The bridge current was at all times kept well below that necessary to stimulate, and excitability was tested at the end of each run.

The phase angles measured for this axon ranged from 70° to 85° which indicates that the membrane impedance is of the polarization type. These phase angles are considerably higher than those found for nerve bundles from the same animal, so it seems very likely that part of the low phase angles found for nerve bundles may be due to a statistical distribution of fiber diameters and membrane capacities. In several cases, impedance runs were taken both before and immediately after the fiber lost excitability, and none of the impedance characteristics of the axon changed when this occurred. Some time later, however, the membrane impedance dropped to zero which indicated the death of the cell. Membrane capacities found for this axon average 0.42 μf/cm.^{2} at 100,000 cycles, which is in good agreement with the values previously found for nerve bundles.

Electric impedance of suspensions of unfertilized and fertilized Arbacia eggs. K. S. Cole and J. M. Spencer.

The alternating current resistance and capacity of suspensions of *Arbacia* eggs in sea water, measured at frequencies from one thousand to ten million cycles per second, give average membrane capacities, with 90° phase angles, of 0.84 μf/cm.^{2} for the unfertilized, and 3.5 μf/cm.^{2} for the fertilized eggs. Some slightly lower phase angles were probably indications of abnormalities. The previously reported additional capacity element in the fertilized eggs disappeared as the season progressed. It was found that the plasma membrane enclosed volume averaged 1.7 per cent less than the non-conducting volume for the unfertilized egg and 2.5 per cent less for the fertilized egg, while the fertilization membrane enclosed volume averaged 32 per cent greater than the non-conducting volume. Thus the plasma membrane lies very close to the non-conducting membrane in both the unfertilized and fertilized egg, and the fertilization membrane is practically perfectly conducting. It is then probable that, on fertilization, the plasma membrane capacity increases to some four times its unfertilized value.

Electric impedance of single Arbacia eggs. K. S. Cole and H. J. Curtis.

The end of a two or three-mm. thin-walled glass tube is heated until it closes down to a short capillary about 50 μ in diameter. The tube is partially immersed in sea water, and when an egg, dropped in the upper open end, settles to the top of the capillary, the water level in the tube is raised until the egg is pushed into the middle of the capillary. Impedance measurements are then made between electrodes placed in the tube and in the outside solution. The low frequency resistance of a 48 μ tube rose from 24,000 ohms, when filled with sea water, to 840,000 ohms with an unfertilized egg in place. This increase might be due to a membrane resistance of 20 ohm cm.^{2} but this value is no more than a lower limit since a layer of sea water 0.25 μ thick between the egg and the glass would produce the same result. The low frequency resistance for a fertilized egg was equivalent to a 4.2 μ space, which is larger than the membrane elevation. The observed low frequency capacities and the higher frequency data give average membrane capacities of 0.8 μf/cm.^{2} for the unfertilized, and 2.8 μf/cm.^{2} for the fertilized eggs. These results are in agreement with these obtained from suspensions and the technique may be used for several problems which are not otherwise possible.

The effect of NaCl on potentials in Nitella. Samuel E. Hill.

The normal action current in *Nitella* requires about 15 seconds for completion, including recovery. After the cells have soaked for 30 minutes or more in 0.01 M NaCl the action currents may become very brief, lasting not more than 1 or 2 seconds. The form of the action curve changes, showing 1 peak instead of 2, and the amplitude is usually less.

After 24 hours in 0.01 M NaCl the action curve tends to become normal again.

The cells show no signs of injury after 24 hours or more in 0.01 M NaCl.

The coalescence of a plant cell with oil drops. M. J. Kopac.

The young aplanospores of *Valonia ventricosa* are essentially naked protoplasts and coalescence with oil drops readily occurs. As the aplanospores become older, the tendency to coalesce with oil drops becomes decreased. Coalescence with oils of a high interfacial tension against sea water (paraffin oil, tension *ca.* 40 dynes^1 cm.$^{-1}$) is inhibited in aplanospores over 3½ hours old. This decreasing tendency to coalesce with oil drops is believed to signify the building up of extraneous coats by the protoplast. Ultimately the aplanospores become coated with a cellulose wall.

In aplanospores about 1½ hours old, coalescence with low tension oils (oleic acid in paraffin oil, tension *ca.* 3 dynes^1 cm.$^{-1}$) occurs rarely. A small drop of this oil may be placed in contact with an aplanospore without coalescence occurring. If a few seconds later a small drop of a higher tension oil (oleic acid, tension *ca.* 10 dynes^1 cm.$^{-1}$) is applied to the opposite side, coalescence between it and the aplanospore immediately takes place. From ½ second to several minutes later, the first drop snaps into the aplanospore. In these young aplanospores no cellulose cell wall has been formed. The inhibition of coalescence with a low tension oil may be due to the preliminary solidification at the cell surface prior to the formation of a cellulose wall. Coalescence with oleic acid apparently induces a peripheral disorganization at the cell surface which then permits the protoplast to coalesce with a low tension oil. This disorganization in the case of the aplanospore may actually be a disintegrative action at the cell surface. Additional evidence for this point is shown by the release of chloroplasts from the protoplast following coalescence with the two drops. These investigations were started at the Tortugas Laboratory this summer and are being continued at the Marine Biological Laboratory.

The influence of length, tension, and tone upon the birefringence of smooth muscles (Phascolosoma and Thyone). Ernst Fischer.

The retractor muscles of *Phascolosoma* and *Thyone* respond to direct stimulation with a twitch-like contraction. After indirect stimulation involving the ganglion the quick contraction of both muscles is followed by a sustained tonic contraction. Besides this "contractile tone" both muscles show marked "viscosoidal tone." A muscle stretched by a load is constantly lengthening, and when the muscle is released later on, it shortens only to a small extent. By alternatingly loading and releasing a muscle with increasing weights, the birefringence of the muscle can be measured for the same muscle length under no tension and under a well-determined tension. When no tension is exerted, the birefringence of the muscle is about proportional to the square root of the length, as found by Bozler for a smooth snail muscle under comparable conditions. For the same muscle length under tension the birefringence is higher, the increase being proportional to the tension present,—a true "photoelastic effect." In consequence, when a muscle is stretched quickly, the birefringence increases at a steadily growing rate until suddenly the birefringence diminishes and the muscle tears through.

Under isometric conditions the tonic sustained contraction increases the double refraction under all conditions, while for the twitch-like contraction, as shown previously, the direction of the birefringence change is dependent on length and tension. This indicates that "normal contraction" and "tonic contraction" are fundamentally different processes. For both muscles investigated the same results were obtained with the only difference that in *Thyone* the birefringence is merely 60 per cent of that in *Phascolosoma.*

The mechanism of salt penetration in Amoeba—some micromanipulative data. Samuel A. Corson.

Addition of inorganic acids (to a pH of 4.5 or lower) to K or Na salt solutions in which the amoebae were immersed prevented the decrease in protoplasmic viscosity and the cessation of movement which occurred when *Ameba proteus* (*Chaos diffluens*) was immersed in neutral or alkaline solutions of these salts. The same acids also prevented the marked increase in protoplasmic viscosity produced by immersion in neutral or alkaline Ca salt solutions. Utilizing the Chambers micromanipulator and a new method which permits quantitative injections (the method consists essentially of enclosing the solution between a layer of heavy and one of light oil and measuring the quantity of solution drawn into the pipette by means of a calibrated ocular micrometer) it was shown that while KCl injections produced the same effects as in the immersion experiments (a fact observed previously by R. Chambers), K_2SO_4 injections failed to liquefy the protoplasm though they did inhibit locomotion. These effects, just as the effects of $CaCl_2$ injections, were not influenced by acidification of the injected solutions (to a pH of 4.2–3.0). Since in the immersion experiments the K effect was the same irrespective of the anion used, these results support the previously suggested hypothesis that the plasma membrane of this amoeba is selectively permeable to cations and relatively impermeable to anions.

The efficiency of monochromatic ultraviolet radiation in the activation of Arbacia eggs. Alexander Hollaender.

During an investigation of the effects of ultra-violet radiation on the eggs of *Arbacia punctulata,* it was observed that when the eggs are exposed for as short a time as 1/10 of a second to the entire radiation of a water-cooled high pressure quartz capillary mercury vapor lamp, a large percentage of the eggs went through one or more cleavages without fertilization. Exclusion of the infra-red did not inhibit while exclusion of the radiation below 3,000A inhibited the effect.

The eggs were then irradiated with measured quantities of monochromatic radiation of nine different wave-lengths from 2,260 to 3,650A. Special care was taken to develop a method which would make certain that not only each egg within the dish but each part of each egg received an equivalent amount of energy. This was done by rotating a small dish in which the eggs were suspended in 3 mm. of sea water and blowing an air current against the water surface. The eggs were removed after irradiation to a larger volume of sea water and kept at 24° and 10° C.

Three types of controls were handled in each series of experiments: (1) unirradiated, unstirred eggs, (2) eggs stirred in the usual manner but protected against the radiation, (3) sea water irradiated with the wave-lengths most effective in producing activation to which unirradiated eggs were afterwards added. In none of these controls could any activation be recognized if the original eggs were in good condition.

The energy was measured with a standardized vacuum thermopile-galvanometer set up and the incident energy per egg calculated taking into account the total energy entering the dish, the time of exposure, the diameter of the egg and the fact that the eggs were exposed interruptedly ignoring for the present the energy reflected and scattered by the eggs.

Three to five hours after irradiation a high percentage of the eggs (up to 98 per cent) were found activated (one or more cleavages), if certain wave-lengths and energies per egg were used. The wave-length found most effective was 2,260A, and the efficiency decreased with the increase in wave-length, becoming negligible around 2,500A; 0.13 to 0.25 erg of incident energy per organism at 2,260A produce the highest percentage of activation. A plot of the percentage cleaving against the energy applied for each effective wave-length shows a typical S-shaped curve, a definite plateau, and finally a decreasing rate of cleaving with further increase of energy.

A plot of the efficiency of radiation against wave-lengths with its most pronounced maximum at 2,260A and its low efficiency at 2,600A suggests interesting comparisons with the ultra-violet inactivation spectra of urease and the virus of typical tobacco mosaic, and the absorption spectra of other protein-like substances.

Activation of centrifuged whole eggs of Arbacia and their fractions by monochromatic ultra-violet radiation. Ethel Browne Harvey and Alexander Hollaender.

Whole *Arbacia* eggs which have been stratified and elongated by centrifugal force (10,000 x g for 3 minutes) are activated by the same ultra-violet radiation as the whole uncentrifuged eggs; full arc 1/10–5 seconds, monochromatic 2,350–2,480A for 2–8 minutes. They behave in exactly the same way as when activated by other parthenogenetic agents such as hypertonic sea water but pass through only a few cleavages.

White half-eggs (nucleate) are activated by the same radiation but are much more sensitive to slight variations from an optimum treatment, and fewer cleave.

Red half-eggs (non-nucleate) are activated by the same radiation and also by a band of longer wave-length, 2,650–3,050A for 4–12 minutes, which does not affect the whole eggs and the white halves. Fertilization membranes are formed, some large asters occur, the egg often becomes aspherical and somewhat amoeboid. A notch frequently appears at the equator of the more spherical red halves, indicating the beginning of a cleavage plane, and this usually completes itself. Stages with 8–12 cells have been observed usually unequal in size. There occur later on some eggs filled with many small asters, a possible precursor to a blastula.

Yolk quarters (non-nucleate) are activated in just the same way as the red halves and by the same wave-lengths and dosage, and some 8–12-celled stages have been observed. The pigment quarters (non-nucleate) show some evidence also of being activated, since they form an ectoplasmic layer and become somewhat amoeboid.

Ultra-violet radiation, therefore, acts upon the whole eggs and their fractions obtained by centrifugal force exactly like other parthenogenetic agents such as hypertonic sea water. Since the radiation affects the non-nucleate fractions as well as the nucleate, the action must be on the cytoplasm, but since many abnormal and irregular mitoses are observed in stained sections of later cleavages, the action must be also on the nucleus.

The cytology of Arbacia punctulata activated by monochromatic ultra-violet radiation. B. R. Nebel, Ethel Browne Harvey and Alexander Hollaender.

Unfertilized eggs of *Arbacia* treated in the summer of 1936 with the full output of a high pressure mercury vapor lamp for a few seconds, showed activation in all the eggs and fairly normal first cleavages following the normal nuclear changes in 80 per cent of the eggs, but the cleavage was delayed by two or more hours. A very characteristic feature of late prophase and metaphase stages in later cleavages as shown in prepared sections is the presence of small rings or spheres among the chromatic material. Occasionally the spindles of more than two plates appear united at various angles. Large cytasters are observed. Regular dicentric spindles, typically non-astral, occur but the most frequent type is a monaster surrounding the chromosomes, which as the division proceeds appears to form two polar half-asters. The eggs developed usually only to about an 4 to 8-cell stage.

Unfertilized eggs treated in the summer of 1937 with measured intensities of monochromatic ultraviolet of 2,350 and 2,260A for 4 to 6 minutes show up to 98 per cent activation indicated by a fertilization membrane and ectoplasmic layer and after 3–4 hours cleavage. Nuclear divisions are monastral and irregular, normal dicentric asters not being formed. The first division is frequently of the restitution type leading to diploidy without cell or nuclear division. The length of the spindle is diminished. During subsequent stages the products of successive chromatic multiplication may be separated. Thus irregular blastomeres may be formed containing no chromatin, approximately haploid, diploid or polyploid nuclei. The average activation does not produce more than 4 to 6 nuclei containing approximately variable multiples of the haploid chromosome number, distributed irregularly in 2 to 8 asymmetrical blastomeres.

Unfertilized eggs treated with the same and larger total energy given as ultraviolet $\lambda = 2{,}480$, 2,650 and 3,050A showed no marked activation.

Fertilized eggs treated with ultraviolet of the short wave-length (2,260 and 2,350A) soon after insemination showed slight inhibition. Fertilized eggs treated with ultraviolet of longer wave-length (2,650 and 3,050A) showed marked inhibition. In both treatments occasional nuclear irregularities were observed.

The relationship of sperm extracts to the fertilization reaction in Arbacia.

John A. Frank.

A specific egg-agglutinin, previously reported, is present in the filtrate from boiled *Arbacia* sperm suspensions. This substance is present in the fat-free residue on extracting sperm suspensions with alcohol and ether. It is not found in the lipid extract.

Eggs fertilized in sperm extracts show a marked drop in fertilizability when compared with eggs fertilized in sea water. Experiments were performed to determine whether this inhibition of fertilization is due to the action of sperm extracts on the egg alone, spermatozoön alone, or on both gametes.

Sperm suspensions exposed for varying lengths of time to sperm extracts exhibit a marked loss in fertilizing power. Sperm extracts thus block fertilization by a rapid direct action on sperm.

The fertilizability of jellyless eggs exposed to sperm extracts and subsequently fertilized with fresh sperm decreases markedly. Sperm extracts therefore exert an inhibitory effect on the cortex.

When sperm extract is added to egg water containing fertilizin, the mixture will not agglutinate sperm. Some substance in the sperm extract has inactivated fertilizin. Sperm extracts inactivate fertilizin in definite quantitative proportions. The capacity for fertilizin inactivation varies directly with the concentration of sperm extract. Thus sperm extracts contain a substance which resembles Lillie's anti-fertilizin.

On ageing, the fertilizing power of sperm suspensions is lost concurrently with the capacity of extracts of these suspensions to inactivate fertilizin and to agglutinate eggs.

Evidence at present indicates that sperm extracts contain substances related in some way to the fertilization reaction.

Stimulation and nuclear breakdown in the Nereis egg. L. V. Heilbrunn and Karl M. Wilbur.

Recent studies of stimulation in diverse types of protoplasmic systems have indicated that one of the primary effects is a breakdown of a calcium proteinate gel in the cell cortex and a release of free calcium into the cell interior. As yet these studies of stimulation have thrown no light as to the rôle the nucleus may play when a cell is stimulated to divide. In the egg of the worm *Nereis,* various types of stimulating agents cause a breakdown of the germinal vesicle. Thus, such an effect is produced by heat, ultraviolet radiation, and Roentgen rays, as well as by various chemicals. If our theory is correct, one of the initial steps in the series of processes that result eventually in nuclear breakdown is a calcium release from the cortex of the cell. Our experiments lend support to this theory. If *Nereis* eggs are exposed for 6 or more minutes to an isotonic citrate solution, subsequent treatment with ultraviolet radiation causes no nuclear breakdown; although on return to sea water the eggs again show a typical response following irradiation. Similarly previous treatment with citrate prevents the nuclear breakdown which otherwise occurs very beautifully when eggs are placed in isotonic solutions of sodium or potassium chloride. The sodium and potassium ions appear to be capable of provoking a release of calcium ions from the cell cortex, and these calcium ions induce changes which eventually lead to a breakdown of the nuclear membrane.

The movement of the egg nucleus in relation to the sperm aster in Lytechinus and Echinarachnius. Edward L. Chambers.

The observations indicate first that the egg nucleus is moved to the center of the sperm aster by centripetal currents of cytoplasm. This is in conformity with the early observations of Conklin. He held that the approach and union of the two nuclei were determined by protoplasmic currents in the oöplasm.

The granules in the cytoplasm move along with the egg nucleus.

The curvature of the path of the egg nucleus is caused by the continual change in direction of the cytoplasmic currents due to the progressive movement of the sperm aster towards the center.

The increasing acceleration of the egg nucleus indicates the existence of more and more intense centripetal currents of cytoplasm as the pronucleus migrates into the aster.

These observations indicate, second, that the aster is a jellied mass. This confirms R. Chambers' conclusion.

The diminishing acceleration of the egg nucleus as it moves down the ever-narrowing cytoplasmic path extending from the margin to the center of the aster demonstrates a resistance to movement due to the presence of a jellied material around the path through the aster.

The deformation (ellipsoidal) of the egg nucleus as it moves along this path demonstrates that the path is gradually tapering cone of fluid cytoplasm in the jellied mass of the aster.

The physical state of the wall of the furrow in a dividing cell. Robert Chambers.

Evidence is accumulating that the wall of the furrow is solid and constitutes the most solid part of the cortex of the cell. The advance of the furrow displaces the interior toward the poles which bulge because of the relative weakness of the polar cortex.

No symmetrical arrangement of the surface seems to be essential since strands of cortical protoplasm may be dragged out either at the equatorial or polar regions without affecting the cleavage. The strands at the equatorial region are much stiffer than those at the poles. In tissue cultures fibroblasts often retain extended strands especially at the poles while the furrow forms at the equator.

Conditions within the cell interior need not affect the division once cleavage is under way. A dividing epithelial cell sometimes shows a rhythmic back-and-forth flow of the internal contents through the narrowing stalk connecting the two daughter cells. This ceases only when the constriction of the stalk has completed the division. In the sea-urchin egg undergoing division in calcium-free sea water, the contents of one blastomere, when torn, pour out while the furrow continues to deepen. The continued pinching-down of the furrow on the connecting stalk frequently rescues the other blastomere from disintegration.

The thickness of the cortex of the advancing furrow and the force with which it advances has been determined by injecting an oil drop into the equatorial region. The oil drop tends to come to lie in the central region at the equator so that the advancing furrow closes down on it and constricts it in two. This occurs when the surface of the floor of the furrow is some distance from the surface of the oil-drop.

The advance of the wall of the furrow must be considered as a growth phenomenon—material being added progressively to the gelated cortex of the furrow analogous to the apposition of material along the plane of division of a plant cell.

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Chromosome studies in sundew (*Drosera*). A. Orville Dahl.

For purposes of comparison with chromosomes in certain members of the Saxifragaceae, cytological material of *Drosera filiformis* Raf., *D. longifolia* L., and *D. rotundifolia* L. has been locally collected. A preliminary examination of root-tip meristem in aceto-carmine demonstrates 20 chromosomes in *D. filiformis* which is consistent with Levine's (*Mem. N. Y. Bot. Gard.*, 6: 125–147, 1916) report of 10 chromosomes in pollen mother-cell material from Lakehurst, N. J. Rosenberg (*Ber. der Deutschen Bot. Ges.*, 21: 110–119, 1903) found 20 chromosomes in cells of root-tips, stems, leaves, and flowers of *D. rotundifolia* collected in Germany, Norway, and Sweden. A comparative study of the early somatic metaphase in *D. filiformis* from Mashpee, Mass. and *D. rotundifolia* from North Falmouth, Mass. shows that the chromosomes of the former are approximately 1.49 times longer and 1.38 times wider than those of the latter. The metaphase chromosomes are of comparatively small size, those of *D. rotundifolia* being about 1.90 μ in length while those of *D. filiformis* are 2.82 μ in length. A visibly four-parted structure, with the distance between the half-chromatids 0.3 μ to 0.4 μ, could be detected at mid-prophase, late prophase (at which time the nucleus has a diakinetic appearance), early metaphase, and late telophase.

Mitosis in the giant amoeba, Chaos chaos Linnaeus. M. Catherine Hinchey.

Chaos chaos is from 50 to 500 times as large as *C. diffluens* and has from 50 to over 1,000 discoid nuclei. The chromatin granules are arranged in a layer underneath the nuclear membrane in the living amoeba.

When mitosis begins, the nuclei become spherical and the chromatin granules congregate in a thick equatorial plate. Then most of the granules seem to disappear, leaving a spherical nucleus, with chromatin granules distributed in a thin plate. What appear to be spindle fibers become visible at this stage. These fibers can be seen in the living amoeba under the micro-compressor.

Following this stage, the nucleus shortens along the polar axis and becomes wider at the equator, so that a ladder of fibers is seen, in optical section, with the chromatin distributed along the mid-points of the ladder-rungs.

The chromatin then separates into two plates which move along the spindle fibers toward the poles, until the plates are three times their diameter apart. At this stage fibers can be plainly seen in both stained and living material, extending between the plates and from the plates to the poles.

Protoplasmic streaming next moves the plates apart. The fibers between the plates become twisted and disappear, but the fibers going to the poles persist for some time longer. The chromatin plate becomes thinner, and so homogeneous that it is extremely difficult to follow in living material, but in fixed material, a concentration of chromatin occurs along the inner edge of the plate. The daughter nuclei next become wider and more granular. This process continues by gradual steps until the interphase stage is reached.

Cytoplasmic division—usually into 3 daughters—occurs during the reorganization of the daughter nuclei into interphase nuclei.

The striking features of the nuclear division are: 1. All the nuclei divide at the same time. 2. Practically every stage of mitosis can be seen in the living amoeba under the micro-compressor. 3. Although the total number of nuclei doubles during mitosis, cytoplasmic division usually results in three daughters.

Some effects of oxygen on polarity in Tubularia crocea. James A. Miller.

A chamber was constructed by means of which the two ends of *Tubularia* stems could be exposed to different agents or to different concentrations of the same agent. This consisted of a double chamber with a partition which separated the solutions but which had perforations through which the stems could be passed. Using this apparatus preliminary studies were made upon the effects of high and low oxygen tensions on polarity. By placing the stems in alternating orientations each experiment served as its own control. Oxygen determinations by the Winkler method were made in all but preliminary experiments.

When oxygen was bubbled on one side of the partition and boiled sea water was placed on the other, hydranths developed only on the side with high oxygen. One half of these were distal and the other were proximal hydranths. Similar results were obtained when oxygen was bubbled on one side of the partition and standing sea water (with 4.1 to 5.0 cc. O_2 per liter) was on the other. That these results were caused primarily by the oxygen differential and not by a possible accumulation of carbon-dioxide was demonstrated when nitrogen was bubbled on one side of the partition and oxygen on the other. Here again there was a reversal of polarity in all stems with their proximal ends exposed to the oxygen.

The importance of circulation of the medium to sessile forms such as *Tubularia* was illustrated by experiments in which 95 per cent of the stems developed proximal hydranths in running aerated water when the distal ends were exposed to standing water, while only 6.3 per cent developed proximal hydranths when the conditions were reversed. Oxygen determinations in two of these experiments showed a difference of only 0.1 cc. per liter in each case.

Some effects of strychnine on reconstitution of hydranth primordia in Tubularia crocea. Faith Stone Miller and James A. Miller.

Miller (1937) found that pieces of planarians regenerating in strychnine showed no evidence of stimulation. Since in *Tubularia* the size and time of development of hydranth primordia can be measured, this form was used to continue the study of the effects of strychnine.

The behavior of unoperated individuals placed in solutions of strychnine sulphate in sea water was undistinguishable from that of controls anesthetized by magnesium sulphate.

In reconstitution experiments stems selected for uniform appearance and diameter were used. Ten-millimeter pieces were cut with the distal end five millimeters from the base of the hydranth. Continuous exposure resulted in a decrease in size of hydranth primordia and increase in time of development. Concentrations used ranged from M/20,000 which showed very little effect to M/5,000 in which few stems survived. Temporary exposures to M/1,000 for periods of ½ to 8 hours showed similar but less consistent results. The frequency of the occurrence of bipolar forms was decreased in strychnine and none developed in the higher concentrations.

The results obtained with strychnine on *Tubularia* are similar to those with inhibitory agents and indicate that it produces a definite depression. By bubbling oxygen through the solutions it was possible to antagonize the strychnine effect.

The life cycle of Moniezia expansa. Horace W. Stunkard.

Anoplocephaline cestodes are common parasites of herbivorous animals and one species is recorded from man. They are worldwide in distribution, and the group has been intensively studied for more than fifty years. The final hosts harbor sexually mature worms in their intestines, eggs of the parasites are voided with the feces of the hosts, but what occurs in the interval before the cestode reappears in the intestine of the primary host has remained quite unknown.

Stunkard (1934) published results of experiments which demonstrated that the final hosts could not be infected with eggs of the parasite and that an intermediate host is necessary for the completion of the life cycle.

Experiments have been continued, using species of *Moniezia* from sheep and *Cittotaenia* from rabbits. Various minute, terrestrial invertebrates, chiefly insects, have been used in attempts to discover the intermediate hosts of these cestodes. In the spring of 1936, tyroglyphid mites were fed eggs of *Moniezia* and onchospheres were recovered from the body cavity three days later. The structure, habits, and life history of these mites indicate that they would not be suitable intermediate hosts of *Moniezia*. The oribatid mites, however, appeared to be admirably suited, and representatives of this family were employed. *Galumna sp.* are abundant in regions where *Moniezia* occurs and specimens of this mite were collected from areas in which there were no sheep. These mites were fed eggs of both *Moniezia* and *Cittotaenia* and onchospheres of both species were recovered from the body cavity. During the past year thousands of *Galumna sp.* have been fed eggs of *Moniezia expansa* and a series of developmental stages, from the onchosphere to the mature cysticercoid, have been recovered from them.

A new method for studying the pH of the intercellular substance in the living mammal. Richard G. Abell and Eliot R. Clark.

This method involves the installation of phenol red within a transparent moat chamber in the ear of a rabbit. Such a chamber contains a thin space, called the 'bay,' into which living tissue, continuous with the subcutaneous tissue of the ear, grows through two small entrance holes at the proximal end. The bay has a glass bottom and a mica top, and is only 50μ to 100μ deep. Consequently the arterioles, capillaries, and venules, and other constituents of the tissue within it can be seen clearly with the microscope. At its distal end the bay opens directly into a reservoir, called the 'moat.'

Following the introduction into the moat of a 0.4 per cent solution of phenol red, made isotonic with rabbit's blood by the addition of NaCl, the indicator diffuses into the bay, and there colors diffusely the intercellular substance of the tissue for a distance of approximately 1 mm. proximal to the most advanced capillaries. It is not concentrated by the cells, and it is not toxic.

The color of the indicator in the intercellular substance of tissue with an active circulation is pink, the shade of pink matching that of a phosphate buffer at pH 7.2, to which phenol red has been added, seen with a microscope in the counting chamber of a hemocytometer under the same conditions of illumination as used for the tissue.

When the circulation is cut off, by compressing the main artery of the ear, the color of the intercellular substance changes to the orange-yellow of a buffer at pH 6.8 within 10 to 15 minutes, indicating accumulation of acid metabolites. Within 1 to 2 minutes after the artery is released and the circulation once more becomes active, the color of the intercellular substance changes from orange-yellow back to pink.

By means of the present method, the pH of the intercellular substance can be studied under a variety of experimental conditions.

The behavior of living mammalian arterioles, capillaries, and venules when exposed to CO_2. Richard G. Abell and Eliot R. Clark.

The experiments to be described were performed in a transparent moat chamber in the ear of a rabbit. The behavior of the vessels was studied with the microscope, and changes in the pH of the intercellular substance detected by means of the indicator method presented above.

When CO_2 is passed through the moat, the color of the phenol red in the intercellular substance of the tissue in the bay turns from pink (pH 7.2) to yellow (pH 6.8–6.6). No increase in stickiness of the endothelium occurs when the amount of CO_2 employed is small. If the endothelium is sticky toward leukocytes before CO_2 perfusion is started, it reverts to a state in which the leukocytes roll freely along the vessel walls.

No increase in the diameter of the arterioles, capillaries, or venules occurs as long as the circulation continues to pass through them. The arterioles in these experiments were not supplied by nerves.

The color of the indicator in the intercellular substance of the proximal tissue turns from pink to yellow more rapidly when the circulation is sluggish than when it is rapid. Such color change is followed shortly by thickening and vacuolization of the endothelium of the arterioles.

If CO_2 perfusion is stopped at this stage, the vacuoles disappear, and the endothelium resumes its normal thickness. If perfusion is continued, a marked increase in permeability of the arterioles, and also of the capillaries and venules, occurs. The plasma passes through the walls of the vessels, leaving within their lumina only the formed elements of the blood. During this process extensive crenation of erythrocytes occurs. In vessels containing concentrated cells, the flow of blood is blocked.

No increase in diameter of the arterioles, capillaries, or venules occurs at the time of onset of plasma hemorrhage, but may take place shortly thereafter, indicating a softening of the endothelium. These changes are reversible if CO_2 perfusion is stopped when plasma hemorrhage first occurs.

The control of peripheral circulation in the mammal. Eliot R. Clark and Eleanor Linton Clark.

It has been possible, in transparent chambers introduced in the rabbit's ear, to watch the mode of formation and behavior of extra-endothelial cells, with the following results:

Fibroblast-like cells from outside the endothelium become flattened out on the wall of the capillary at a very early stage in capillary formation—often during sprout formation. If the vessel remains a capillary, there may be no increase in their number and they may remain permanently as sparsely distributed inert, oval cells. If the capillary becomes a portion of an artery the number of outside cells increases rapidly—in part at least by mitotic division—the axis of the cells changes quickly from a longitudinal to a transverse position and muscle cells develop which show typical contractility in case they receive a nerve supply.

If the capillary becomes a part of a venule or small vein, the number of adventitial cells increases slightly, their long axis remains longitudinal and they do not develop contractility.

Persistent observations of the living vessels made under a great variety of conditions has corroborated earlier findings that, in the mammal, neither the endothelium nor the adventitial cells, as found on the capillary or venule, manifests active contractility. The control of the peripheral flow resides in the muscle cells of arteries, arterioles and certain of the veins—providing the muscle cells are under nerve control.

While there may be changes in the caliber of capillaries and bulgings into the lumen of endothelial nuclear thickenings or of adventitial cells, all such changes are apparently passive, secondary to a variety of factors, chief of which are changes in internal pressure and rate of flow, produced by contraction or dilatation of supplying arteries or arterioles, and changes in outside pressure occasioned either by variations in the amount of fluid accumulation in the intervascular spaces or by the elasticity of the enclosing wall.

The structure of the liver lobule. Louis Loeffler.

The liver of the pig is the only one which shows clearly defined lobules. By that is meant that the lobules have a connective tissue membrane separating one lobule from another. The capillaries, also, of an individual lobule are separated from the capillaries of the adjacent lobules. All the other livers of mammals, reptiles and fishes, so far as has been investigated, show no separating membranes and also show anastomosing capillaries throughout the whole organ. Nevertheless, one is justified to speak of liver lobules, because the vessels, situated in regular distance from one another, form figures similar to the pig liver lobules. It is shown, however, that there are no so-called sub-lobular veins, because such veins, next in size to the central veins within each lobule, function as central veins quite the same. Hepatic veins come to lie outside the lobules, not before. The hepatic veins reach a diameter half or much more than that of a liver lobule. A collecting lobe of about 6 or 8 lobules around a sub-lobular vein as usually found in the diagrams of anatomical textbooks does not exist or is quite arbitrary. The pig's liver should be explained on the basis of a physiological liver cirrhosis.

A preliminary note on the innervation of the swim-bladder of the sea-robin. John B. Gaylor and Ernst Scharrer.

This communication deals with the peripheral innervation of the sea-robin as investigated by Dr. Gaylor at Woods Hole; a combined paper will be published later when Dr. Scharrer has worked out the central connections.

The swim-bladder of the sea-robin consists of a two-lobed sac in the abdominal cavity. It possesses intrinsic skeletal muscle which subserves the function of noise production and, in the interior of the cavity, a gas gland which is in the form of a "rete mirabile" covered with secretory epithelium. A branch of the vagus on either side affords a motor supply to the striated musculature. Free endings, knob endings and ring terminations have been observed. There is no apparent difference in the mode of termination in the swim-bladder muscle from that of the usual somatic musculature. Ganglia in the striated muscle are sparse; the disparity between the wealth of fiber and the number of ganglia argues a double innervation to the skeletal muscle—one direct from the vagal nucleus and one relayed through peripheral ganglion cells. The presence of muscle-spindle organs in the region where the muscle takes origin from the fibrous sac is suspected but not yet definitely established.

The "rete mirabile" contains fine non-medullated fibers which enter along with the vessels and which appear to be sympathetic. Between the vessels there is a large number of multipolar ganglion cells which are presumably parasympathetic relays.

The origin and development of the thyroid in Eleutherodactylus, an anuran with no tadpole stage. W. Gardner Lynn.

The Jamaican tree-toad *Eleutherodactylus nubicola* lays its eggs on land and the young hatch after about twenty-four days with a definitive body form. During the embryonic development some of the larval characters which are usually found in frogs appear very transiently but others, such as the formation of external and internal gills, are entirely lacking. A study has been made of the thyroid in embryos preserved at twenty-four-hour intervals throughout the period of development. The thyroid takes its origin from the pharynx at the sixth or seventh day and the cells exhibit signs of secretory activity almost as soon as the thyroid anlage is definitely recognizable. Throughout the succeeeding stages there is a steady increase in the amount of stored colloid. Intracellular vacuoles are abundant in the follicle cells at all stages. Vacuolation of the colloid mass is most striking during a period of about four days extending from the tenth to sixth day before hatching. There is no evidence of a sudden release of any large amount of colloid into the blood stream at any time during the embryonic history. The indication is, rather, that a regular release occurs even from the early stages. This would agree with the regular course of bodily differentiation and the absence of any striking metamorphic pattern. However, certain of the unusual features of development in this frog, such as the absence of external gills, cannot be attributed to precocious thyroid functioning. Thus it appears that while the evolutionary changes which have brought about the atypical life history of the species are some of them changes in the development and functioning of the gland complex, still others are changes which cannot be attributed to hormonal influence but are direct alterations in the developmental potentialities of the tissues themselves.

Some effects of mammalian follicle-stimulating and luteinizing hormones in adult female urodeles.[1] Virginia Mayo.

During October adult salamanders (*Triturus viridescens*) were subjected to preliminary tests on their ovulatory response to a single intraperitoneal injection daily of the following mammalian pituitary extracts made according to H. L. Fevold's method: (*a*) physiologically pure follicle-stimulating hormone (F.S.H.); (*b*) physiologically pure luteinizing hormone (L.H.); (*c*) F.S.H. with a trace of L.H.; (*d*) an unfractionated extract containing both F.S.H. and L.H. Each injection contained 1/20 gram-equivalent acetone-dried sheep pituitary powder. The results indicated that fractions containing L.H. induced egg-laying whereas F.S.H. alone was almost entirely ineffective. Injections given both normal and hypophysectomized newts in November–December, May–June, and June–July–August corroborated the October findings.

[1] This work was begun at the Biological Laboratories, Harvard University, and continued at the Marine Biological Laboratory.

Counts of eggs released by groups of 10 animals treated during the breeding season for 40 days gave the following: hypophysectomized L.H.-injected animals ovulated 801 eggs; normal L.H.-injected, 582; hypophysectomized F.S.H.-injected, 18; and normal F.S.H.-injected, 25.

At the onset of the June–July–August series the newts' ovaries were almost completely emptied of large eggs. By early August the ovaries of F.S.H. and F.S.H. + L.H.-treated animals were filled with yolked eggs, while those of L.H.-treated individuals were only slightly stimulated. Average ovarian weights of 7–14 animals were: untreated controls, 28 mg.; hypophysectomized + L.H., 69 mg.; normals + L.H., 88 mg.; hypophysectomized + F.S.H., 119 mg.; normals + F.S.H., 134 mg.; hypophysectomized + F.S.H. and L.H., 150 mg.; and normals + F.S.H. and L.H., 173 mg.

On the basis of these results it seems that F.S.H. brings about a striking enlargement of the ovary while the L.H. is primarily responsible for egg release

The relation of melanophore responses to vascular disturbances. G. H. Parker.

It is difficult to cut nerves in experiments on melanophore control without at the same time cutting blood-vessels or at least introducing vasomotor disturbances. Does the stimulation of melanophore nerves thus brought about excite vasomotor changes which in turn excite responses in melanophores or do the nerves act directly on the melanophores? In the killifish *Fundulus* and in the catfish *Ameiurus* the melanophores have a double innervation, concentrating nerve-fibers inducing a blanching of the fish through a concentration of pigment in its melanophores and dispersing fibers darkening the fish through a dispersion of this pigment. In the dogfish *Mustelus* there are only concentrating fibers, the dispersion of the pigment being accomplished through a pituitary neurohumor in the blood.

In *Fundulus* and *Ameiurus* when melanophore nerves are cut the dispersing nerve-fibers are stimulated and dark areas or bands result. In these two fishes and in *Mustelus* when the melanophore nerves are stimulated electrically the concentrating fibers are excited and the fishes blanch locally.

One way of finding an answer to the question under consideration is to ascertain whether these responses will occur or not in the absence of an active circulation of blood. To this end the ventral aorta of a given fish was ligated just anterior to the heart and the circulation of blood thus brought to a complete standstill. The melanophore nerves of such a fish were then subjected to electric stimulation or were cut. In all such instances there was either local blanching or darkening according to the stimulus. These responses, though less marked than in normal fishes, showed unquestionably that the complete loss of circulation was not accompanied by a loss of the power of color response and that therefore vasomotor or other vascular changes could not form any essential part in the chain of events between nerve and melanophore.

Some effects of chloroform on the respiratory systems of yeast. E. P. Hiatt and J. K. W. Ferguson.

The rate of reduction of methylene blue in suspensions of yeast is increased by small amounts of chloroform (0.05 per cent by weight). With larger amounts of chloroform, acceleration up to 40 times was obtained. No stage of inhibition was reached with fresh yeast. Dried yeast and yeast extracts, which have a much faster rate of reduction than equivalent amounts of fresh yeast, were retarded by chloroform. The same effects can be demonstrated with other oxidation-reduction indicators, e.g., pyocyanin, thionin, and anthraquinone.

When chloroform was exerting its maximal acceleration, the cytochrome bands showed a characteristic change. Only the C band was visible and it could not be made to disappear on shaking with air. Subsequently, different amounts of chloroform were found to affect the cytochrome spectrum differently. With concentrations up to 0.1 per cent by weight, the time for the appearance of all three bands (rate of reduction), was shortened. At about 0.3 per cent the A band disappeared, the B and C bands appearing slowly and remaining fixed. At about 0.5 per cent the B band disappeared.

Similar but less marked effects were obtained with ether and 95 per cent ethyl alcohol. It is interesting to note that carbon tetrachloride has little effect.

The oxygen consumption of the chloroform-treated yeast, as determined by the Warburg technique, was also greatly increased;—up to 20 times. Maximal acceleration was obtained in a medium of phosphate buffer at pH 6.6. Less effect was noted at pH 7.0 and little or none at 8.5. A similar acceleration was observed with fertilized and unfertilized *Arbacia* eggs.

In view of the prevalent idea that narcotics act by depressing cellular respiration, it seems significant that these accelerating effects on respiration were obtained with concentrations of the same order of magnitude as are effective in producing general anesthesia.

The oxygen consumption of activated and fertilized eggs of Chaetopterus. Jean Brachet.

Unfertilized *Chaetopterus* eggs undergo activation when they are treated with 5 per cent isotonic KCl in sea water; maturation is followed by a series of monasterian cycles leading to the formation of unicellular larvae resembling gastrulae and trochophores (F. R. Lillie's differentiation without cleavage). The oxygen consumption of these activated eggs has been compared with the respiration of unfertilized and of fertilized eggs during 7 hours (Warburg's method). Activation is followed by a considerable drop in the oxygen consumption (49 per cent); and fertilization has exactly the same effect, as was shown first by Whitaker. The O_2 uptake increases then, but at a much slower rate in the activated eggs than in the fertilized ones: while these resume their initial respiratory rate after 3½ hours, the activated eggs need 6 hours to reach that level. The respiration of the unfertilized eggs remains constant for 7 hours. Control experiments showed that isotonic KCl in the concentration of 5 per cent used has no significant effect on the metabolism of *Chaetopterus* eggs: the slope of the curve is not changed if the KCl treated eggs have been repeatedly washed or when KCl is added to the fertilized eggs. The reduced metabolic activity of the activated eggs must thus be linked to either their slower development or to the fact that they remain unicellular.

Influence of respiratory inhibitors on stimulation of metabolism by nitro and halo phenols. M. E. Krahl, Anna K. Keltch and G. H. A. Clowes.

At the 1934 meeting, experiments reported from this laboratory showed that the respiratory stimulation produced by 4,6-dinitro-*o*-cresol in fertilized eggs of *Arbacia punctulata* was progressively inhibited and could be completely abolished by increasing concentrations of potassium cyanide and that the division-blocking effects of the two reagents were additive. During the past three seasons these experiments with cyanide and 4,6-dinitro-*o*-cresol have been extended and similar experiments made with other respiratory inhibitors.

The following concentrations of inhibitors have been found, with eggs in sea water at pH 8, give a suppression of normal respiration which is just measurable (i.e., 5 to 20 per cent): CO, $94CO:6O_2$; 'Amytal' (Iso-amyl Ethyl Barbituric Acid, Lilly), 2×10^{-3} M; malonic acid, 10^{-3} M; and, at pH 6, iodoacetic acid, 10^{-4} M. At these same respective concentrations of the inhibitors there is a progressive decrease in the extent to which the various inhibitors suppress the respiration stimulus by 4,6-dinitro-*o*-cresol, the first two members of the series giving almost complete, and the last two members almost negligible suppression of the stimulated respiration. With the exception of phenyl urethane and malonic acid, with which such experiments were not made, all inhibitors used gave complete suppression of stimulation by 4,6-dinitro-*o*-cresol when tried in sufficiently high concentrations. With partial inhibition by CO or KCN, the optimum stimulation of the residual respiration was produced by concentrations of 4,6-dinitro-*o*-cresol larger than those required in the absence of CO or KCN. With partial inhibition by the other inhibitors, optimum stimulation of the residual respiration was produced by concentrations of 4,6-dinitro-*o*-cresol equal to or less than those required in the absence of inhibitor.

In this series of experiments, the concentration of 4,6-dinitro-*o*-cresol required, in the absence of inhibitors, to give 90 to 100 per cent block to division was 8×10^{-6} M. The concentrations of 4,6-dinitro-*o*-cresol required in the presence of the concentrations of inhibitors mentioned above, which alone gave little or no block to division, were 4×10^{-6} M in CO; 8×10^{-6} M in low oxygen tension; less than 10^{-6} M in KCN; 8×10^{-6} M in malonic acid.

Substituted phenols as inhibitants of the fertilization of Arbacia and of ciliary movement of Arenicola larvae. G. H. A. Clowes, M. E. Krahl and Anna K. Keltch.

It has already been demonstrated for a considerable series of nitro and halo phenols that the point of concentration required for maximum stimulation of respiration corresponds approximately with the point at which cell division is blocked in fertilized sea urchin eggs. In an attempt to throw further light on the nature of the mechanism involved, the concentrations were determined at which certain representative nitro and halo phenols blocked the fertilization of *Arbacia* eggs by sperm and anesthetized *Arenicola* larvae. The block to fertilization and anesthesia of larvae occurred at about the same concentration for each individual compound, but these concentrations differed in certain cases very greatly from the concentrations at which the respiration peak and cell division block occurred.

In the case of 2,4-dinitrophenol and 4,6-dinitro-*o*-cresol, having pK values of 4.1 and 4.4 respectively, the ratios of concentration required for anesthesia to that required for internal cell division block were found to be 137:1 and 228:1. That for 2,4,5-trichlorophenol, having a pK of 6.9, was found to be 19:1, whilst

Concentrations (moles per liter $\times 10^5$) of substituted phenols required to inhibit various physiological functions. pH 8.0.

Compound	I pK	II Ciliary Movement Arenicola	III Fertilization Treated Eggs Treated Sperm	IV Cell Division Arbacia	Ratio III : IV
2,4-Dinitrophenol	4.1	205	410	3.0	137 : 1
4,6-Dinitro-*o*-cresol	4.4	205	205	0.9	228 : 1
2,4,5-Trichlorophenol	6.9	13	26	1.4	19 : 1
o-Nitrophenol	7.2	No effect	No effect	No effect	—
2,4-Dichlorophenol	7.7	51	26	26.0	1 : 1
m-Nitrophenol	8.3	205	205	51.0	4 : 1

those for 2,4-dichlorophenol, having a pK of 7.7 and *m*-nitrophenol, having a pK of 8.3, were found to be 1 : 1 and 4 : 1. It is particularly interesting to note that orthonitrophenol, which although proved to enter the cell, had no effect on cell division or oxidation, had also no effect on the fertilization process or on ciliary movement of *Arenicola*. It appears advisable to refrain from speculation regarding these results until further data are available.

Stimulation of the rate of cell division of Arbacia eggs by carcinogenic hydrocarbons. Anna K. Keltch, M. E. Krahl and G. H. A. Clowes.

As a part of an investigation into the mechanism by which certain polycyclic hydrocarbons produce cancer, a study has been made, during the seasons of 1935 and 1936, of the effects produced by three carcinogenic and two closely related non-carcinogenic hydrocarbons on cell division of fertilized eggs of *Arbacia punctulata*, using each hydrocarbon in the form of its water-soluble choleic acid, these being addition compounds of the hydrocarbon with desoxycholic acid.

Unfertilized eggs were exposed to varying concentrations of each choleic acid in sea water solution for varying periods of time. They were then fertilized, and left in the same respective solutions and at the same respective temperatures during the periods of pretreatment, fertilization and division, with the single exception that at 5° C., the eggs were raised to 15° for approximately five minutes in order to allow fertilization to take place and then returned immediately to 5° C., the controls in every case being subjected to treatment identical with that given the experimental material.

Typical representative results obtained with a five-hour pretreatment are presented in the accompanying table. In these data it is desired to emphasize—(*a*) that the choleic acids of the three hydrocarbons which produce cancer in mice also produce a shortening of division time and that the choleic acids of the two closely related hydrocarbons which produce no cancer in mice do not produce a shortening of division time; (*b*) that, with optimum concentration of 6-methyl cholanthrene choleic acid, there is a progressively smaller relative decrease in the division time as the temperature is raised from 5° to 15° C.

Choleic Acid	Temperature	Minutes to 50 Per Cent First Cleavage		Carcinogenic Activity in Mice
		Control	Minimum with Hydrocarbon	
	° C.			
6-methyl cholanthrene	5	1717	1482	Positive
6-methyl cholanthrene	10	235	221	
6-methyl cholanthrene	15	109	100	
10-methyl-1,2-benzanthracene	15	122	116	Positive
1,2,5,6-dibenzanthracene	15	126	123	Positive
Phenanthrene	15	118	118	Negative
Fluoranthene	15	115	115	Negative

The molecular species concerned in the action of substituted phenols on marine eggs. Albert Tyler and N. H. Horowitz.

In a recently published article (Tyler and Horowitz, 1937) the view was expressed that the substituted phenols penetrate as the undissociated molecule, but exert their respiratory stimulating and reversible block to cleavage effects as the anion. The evidence for this rests on the fact that with any one of these compounds the concentration required for maximum effect varies with the pH of the solution, but the calculated concentration of the undissociated form present is the same at all pH's. This might mean that the undissociated form is the active species. However, when the various compounds are compared, the concentration of undissociated form at maximum effect shows enormous differences. On the other hand, when the comparison is made on the basis of calculated concentrations of the anion present inside the cell the various substances give values of the same order of magnitude. The compounds investigated include the three mononitrophenols, 2,4- and 2,6-dinitrophenol, 2,4,6-trinitrophenol, the three monochlorophenols, 2,4-, 2,5-, 2,6- and 3,5-dichlorophenol, 2,4,6-trichlorophenol and 2,6-dichloro-4-nitrophenol. Four of these; namely, *o*-nitrophenol, trinitrophenol, *o*-chlorophenol, and 2,6-dichlorophenol, show large deviations, but these substances are actually inactive or only slightly active in stimulating respiration.

Extension of the length of fertilizable life to more than twice the control is also obtained with dinitrophenol, confirming the findings reported by Clowes and Krahl. This prolongation occurs at high concentrations giving no respiratory stimulation. Determinations of the temperature coefficient of the respiration of unfertilized eggs shows it to be the same in dinitrophenol as in sea water.

Ovoverdin, a pigment chemically related to visual purple. Kurt G. Stern and Kurt Salomon.

The eggs of the lobster (*Homarus americanus*) owe their green color to a pigment belonging to the class of carotenoid-proteins. According to G. Wald[1] visual purple is another member of this widely distributed group of chromoproteids.

The carotenoid contained in the lobster egg pigment is astacin which is esterified with an as yet unidentified organic acid.[2, 3] This "ovoester" is in turn linked up with a protein of albuminoid character. The name ovoverdin is proposed for the native pigment complex.

Ovoverdin may be obtained in solution by grinding the eggs with sand and extracting them with distilled water. Treatment with an equal volume of saturated ammonium sulfate removes oil globules containing carotine and small amounts of globulins. The solutions may be further purified by repeated precipitation of ovoverdin in saturated ammonium sulfate solution or by dialysis at low temperature.

Ovoverdin has two absorption bands in the visible, at 6,400 and 4,700 A.; in addition it shows the typical protein absorption in the ultraviolet. The molecular weight, according to the rate of sedimentation in the ultracentrifuge as measured by Dr. R. W. G. Wyckoff, is of the order of 300,000. The isoelectric point is at pH 6.7.

Organic solvents and weak acids liberate the orange red carotenoid by virtue of denaturation of the protein carrier. When solutions of ovoverdin or the whole lobster eggs are rapidly brought to 65 to 70° the color turns from grass green to bright orange red. The red form shows increased light absorption at 4,700 A and greatly diminished absorption at 6,400 A as compared with the green form. When the heated material is rapidly cooled, the green color returns. This reversible thermal dissociation is different from an irreversible dissociation which takes place upon longer exposure to these temperatures or upon raising the temperature to the vicinity of the boiling point. At the latter point coagulation of the protein occurs. The sequence of events and a photoelectric study of the color-temperature-time curves indicates a lower energy requirement of the first, reversible stage of dissociation as compared with the later, irreversible stages.

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[1] Wald, G., 1935–36. *Jour. Gen. Physiol.,* **19**: 351.

[2] Kuhn, R., and E. Lederer, 1933. *Ber. Deutsch. Chem. Ges.,* **66**: 488.

[3] Karrer, P., L. Loewe, and H. Huebner, 1935. *Helv. Chim. Acta,* **18**: 96.

It has been suggested[1, 4] that the bleaching of visual purple by light is a disruption of the purple carotenoid-protein complex; the orange carotenoid, retinene, is liberated and the protein is denatured. This assumption, however, is open to the objection that the energy content of the effective wave-lengths of light is smaller than the energy required for inactivation of the visual purple complex. The present observations would suggest that the reversible bleaching of the retinal pigment does not involve a denaturation of the protein component but is rather of the type of the thermal phenomenon here observed. This hypothesis appears to receive support from the fact that the energy levels at which the latter occurs are lower than those at which protein denaturation takes place and secondly that the process observed here is rapidly reversible whereas protein renaturation in general is a time-consuming process and therefore not well suited for the regeneration requirements of visual purple during vision.

The increase of CO_2 and decalcification in certain pelecypods. Louis-Paul Dugal and Laurence Irving.

In three forms of pelecypods, *Venus mercenaria, Ostrea sp.* and *Elleptio complanatus,* the mantle cavity fluid gains CO_2 when they are kept out of water. The change is from 5 or 8 (in fresh ones) to 90 ml. per 100 ml. of fluid (for individuals kept out of water about 5 or 6 days). When the mollusks begin to die, the total CO_2 decreases. Return to water before death restores the CO_2 to normal. The accumulation of CO_2 probably results from a disturbance of respiration.

In *Venus,* which was most carefully studied, the total CO_2 of the M.C.F. increases rapidly. The pH decreases only from 7.4 to 7.2 and the P_{CO_2} increases only from 3 to a maximum of 25 mm. Hg, so that it is evident that the buffering capacity increases.

The shell is eroded during these changes and a few analyses showed that the mantle cavity fluid gained Ca, so that it is easy to guess that the buffering is provided by the solution of $CaCO_3$ from the shell.

The shell erosion is localized in the central inner part; the mantle tissue and no others, gain CO_2. This indicates that the buffering is effected by the activity of a special tissue.

The effect of pH and ionic strength on the activity of carbonic anhydrase. J. K. W. Ferguson and E. C. Black.

The manometric method of Meldrum and Roughton for determining the activity of carbonic anhydrase by following the rate of evolution of CO_2 from a mixture of phosphate and bicarbonate solutions, has been favored, because of its simplicity, for use in physiological and pathological studies. The reaction used in this method takes place in a medium of changing ionic strength and pH. As yet no adequate analysis of the effect of these variables on the activity of the enzyme has appeared.

In this preliminary study the CO_2 output method has been used and consequently the variation of pH was limited to the range from pH 5.7 to pH 8.0. The pH and ionic strength taken as corresponding to a certain activity was the mean pH and ionic strength of the range traversed by the stage of the reaction used in calculating the activity (usually from the beginning to two-thirds completion).

[4] Mirsky, A. E., 1936. *Proc. Nat. Acad. Sci.* (Washington), **22**: 147.

Between pH 6.0 and 8.0 an eight-fold increase in enzymic activity was found, the activity increasing with pH. Between ionic strengths of 0.5 and 0.1 at constant pH a 2.5-fold increase in enzymic activity was found, the activity increasing as the ionic strength decreased.

The distribution of carbon dioxide in dogfish blood. J. K. W. Ferguson, S. M. Horvath, and J. R. Pappenheimer.

After unsuccessful attempts to apply to fish bloods the chemical and kinetic methods of estimating carbhemoglobin, evidence concerning the state of CO_2 in erythrocytes of fish was sought by studying the distribution of CO_2 between red cells and plasma. In this study smooth dogfish (*Mustelus canis*) and a few specimens of spiny dogfish (*Squalus acanthius*) were used. Defibrinated or heparinized blood was used exclusively after it was found that both oxalate and fluoride grossly affected the distribution of electrolytes between cells and plasma.

The bloods were found to fall into two groups as regards total CO_2 capacity. During June and early July the CO_2 capacities at a CO_2 pressure of 40 mm. Hg. were on the average twice as great as in the bloods obtained during late July and August. In the early part of the season the curves were steeper, indicating greater buffer power. This difference could not be attributed to differences in hemoglobin content. The degree of oxygenation has no effect on the CO_2 capacity of dogfish blood.

Usually the concentration of CO_2 in the red cells (per unit H_2O) is greater than in the corresponding plasma. This is different from the situation in mammalian blood and inconsistent with the view that the bulk of the CO_2 in the cells is in the form of active bicarbonate ions. When chloride distributions were studied the contrast was striking. The ratio of intracellular to extracellular concentration of chloride, (r_{Cl}), is always much smaller than the similar ratio for CO_2, (r_{CO_2}). In mammalian blood $r_{CO_2} = \bar{c}\ 1.25 \times r_{Cl}$. In these bloods $r_{CO_2} = 1.7$ to $3 \times r_{Cl}$. If the intracellular CO_2 which is in excess of the amount to be expected from the chloride ratios is assumed to be "non-bicarbonate" CO_2, this "non-bicarbonate" CO_2 is found to comprise about two-thirds of the CO_2 in the red cell or about one-sixth of the CO_2 in the whole blood.

The influence of certain alcohols on the permeability of the erythrocyte. M. H. Jacobs and A. K. Parpart.

Low and moderate concentrations of the so-called indifferent narcotics have frequently been found to decrease cell permeability. Anselmino and Hoenig (*Pflügers. Arch.,* 225: 56, 1930) have reported such an effect in the case of the penetration of human erythrocytes by several non-electrolytes including glycerol. The present study, involving the erythrocytes of a number of species of mammals and of some other vertebrates shows somewhat more complicated conditions. Thus, while n-butyl alcohol in concentrations from 0.0156 M to 0.25 M may greatly decrease the permeability of the erythrocytes, not only of man but also of the rat, rabbit, guinea pig, groundhog and several birds, the opposite effect is obtained with the erythrocytes of the ox, sheep, pig, horse, dog, cat and several reptiles and fishes. In general, these two groups of species are the same as those already distinguished by other properties of their erythrocytes (Jacobs, Glassman and Parpart, *Jour. Cell. Comp. Physiol.,* 7: 197, 1935). In several cases involving decreased permeability, the order of effectiveness of a series of alcohols is: methyl < ethyl < propyl < butyl < amyl. With erythrocytes of the groundhog the effectiveness of *n*-butyl alcohol increases with increasing molecular weight of the penetrating substance in the order: ethylene glycol < glycerol < erythritol < mannitol. As contrasted with glycerol and related substances to which *n*-butyl alcohol increases the permeability in some species and decreases it in others, thiourea, under the same conditions, always shows an increased permeability. The same was found to be true of lipoid-soluble substances such an monoacetin and ammonium salts of weak acids, which by hydrolysis give rise to NH_3 and lipoid-soluble acids. On the contrary, permeability to the ammonium salts of strong acids, where the penetration of the cell by ions is presumably involved, was in all species found to be greatly decreased.

Ionic exchanges of erythrocytes inferred from volume changes. A. K. Parpart, M. H. Jacobs and A. J. Dzienmian.

The exchange of $SO_4^=$ for Cl^- across the erythrocyte surface leads to a volume change of the erythrocyte which may be measured photoelectrically. Cells shrink when placed in isosmotic Na_2SO_4 solutions. The process is reversible since cells which have had the normal Cl^- of their interior replaced by $SO_4^=$ will swell when placed in isosmotic NaCl solutions. The exchange of Cl^- for $SO_4^=$ is relatively slow in comparison with the osmotic shifts that accompany it. The rate of this ionic exchange varies for the erythrocytes of different species of mammals in the order rat > guinea pig > rabbit > beef > pig, cat.

In view of the slowing effect that butyl alcohol, ammonium salicylate and ammonium benzoate have on hemolysis in ammonium chloride it appeared of interest to study the effect of these substances on the exchange of Cl^- for $SO_4^=$. In all of the species studied the above substances were found to slow the rate of this ionic exchange. Control experiments indicated that these substances, in the concentrations used, had little or no effect on the volume of erythrocytes suspended in isotonic NaCl.

Papers Read by Title

Oxygen as a controlling factor in the regeneration of Tubularia. L. G. Barth.

Preliminary experiments in which the distal end of the stem of *Tubularia* was inserted into a glass tube gave complete inhibition of regeneration of this end with a marked increase in rate at the opposite end. This indicated that low oxygen inhibited regeneration.

Following this the oxygen tension of sea water was varied and rate of regeneration was calculated by dividing the length of the primordia in micra by the number of hours necessary for its formation. Results of nine complete experiments agree in showing that from 4.0 to 21.6 cc. of oxygen per liter the rate of regeneration increased linearly with the logarithm of the oxygen tension. Below 3.0 cc. of oxygen per liter the rate of regeneration drops off to 0 at .35 cc. and the shape of the curve depends somewhat on the way in which the experiment is carried out. The lower limit for regeneration is between .35 cc. and 1 cc. of oxygen per liter. At .35 cc. complete inhibition results, which is reversible when stems are returned to high oxygen.

The results are interpreted as showing that regeneration of *Tubularia* is closely dependent on the amount of oxygen which the tissues receive. It is suggested that the perisarc of *Tubularia* is relatively impermable to oxygen and that the stimulus for regeneration is the admission of oxygen due to cutting of the perisarc.

The effects of different drugs on the melanophores of Fundulus heteroclitus. Sinisha B. Bogdanovitch.

In the following experiments the effects on melanophores of different drugs alone and in combination were studied. Isolated scales of *Fundulus heteroclitus* were used and the drugs studied were atropine, pilocarpine, physostigmine, epinephrine, acetylcholine, mecholyl and deuterium oxide. The technical procedures were the same as those described in a previous paper. A summary of the results obtained follows:

Atropine: Expands melanophores and also does so after they had been previously contracted by epinephrine, deuterium oxide, acetylcholine or mecholyl. Ergotized melanophores were also expanded by atropine.

Epinephrine: Contracts melanophores and also does so after they had been previously expanded by pilocarpine, physostigmine or atropine. Ergotized melanophores are, as is well known, expanded by epinephrine.

Physostigmine: Expands melanophores and also does so after they had been previously contracted by epinephrine, acetylcholine, or mecholyl. Well ergotized melanophores are not expanded by physostigmine.

Pilocarpine behaves as physostigmine, but appears to be slightly more effective.

Acetylcholine contracts melanophores and also does so with melanophores previously expanded by physostigmine or pilocarpine. However, with melanophores previously expanded by atropine acetylcholine produces either very slight contractions or none at all. Ergotized melanophores are contracted by acetylcholine. Ergotized melanophores are, of course, already contracted, so that additional contraction is sometimes quite slight. Mecholyl behaves as acetylcholine but the effects appear to be more lasting.

Deuterium oxide contracts melanophores after they had been previously expanded by atropine, pilocarpine or physostigmine. Other effects of deuterium oxide on melanophores were described in a previous paper.

Scales removed from fish and kept in balanced solutions for two hours or more showed an initial expansion in epinephrine followed by contraction. Such a result, while possibly due to a pathological change, may explain contradictory results of previous investigators and offer a clue as to the nature of the innervation of melanophores.

Further investigations on the effect of tissue on different drugs. Sinisha B. Bogdanovitch.

In a former paper it was established that epinephrine and acetylcholine are destroyed by tissue (in this case scales of *Fundulus heteroclitus*), but that deuterium oxide protects these substances from such destruction. Using the same technique as in former experiments atropine, pilocarpine, physostigmine and mecholyl (acetyl β-methylcholine) were similarly investigated with the following results.

Solutions of atropine (sulphate) which had previously expanded melanophores, no longer did so or at most only slightly, after treatment with fish scales for 24 to 48 hours. However, atropine solutions to which scales and deuterium oxide were added for the same length of time, showed no change in expanding melanophores. Physostigmine (sulphate) and pilocarpine (chloride) solutions were not affected by fish scales even after 48 hours. Solutions of mecholyl (chloride) were also practically unchanged by similar treatment.

It thus appears that atropine is destroyed by fish scales as was epinephrine and acetylcholine, but that physostigmine, pilocarpine and mecholyl were not affected. In the former paper it was suggested that the destruction of epinephrine and acetylcholine by fish scales may be due to the action of enzymes such as acetylcholinase and some oxidase. In support of this view I observed that scales which had been heated at 100° C. for 10 minutes did not destroy atropine even after 48 hours. Similar experiments on epinephrine and acetylcholine gave the same results. This thermolability of the "destructive principle" in fish scales for these three substances is consistent with the enzyme hypothesis.

A quantitative study of the staining of marine eggs by neutral red. Barry Commoner.

Unfertilized eggs of *Chaetopterus* and *Nereis limbata* were stained in sea water containing various concentrations of neutral red at 14.0°, 15.5°, 17.0°, 18.4°, and 19.5° . The pH was maintained at 7.6.

Eggs were suspended in sea water acidified to pH 7.6. The density of eggs per ml. of suspension was determined and a known quantity of neutral red added. The same certified stain of 61 per cent dye content was used throughout. Concentrations are expressed as mgm. of this preparation.

Samples were removed periodically from the suspension and centrifuged to concentrate the eggs. The eggs were washed in sea water to remove unbound stain, again concentrated and shaken in 10 ml. of a neutral red extractant. (One volume of N/1 HCl and 9 volumes of 95 per cent ethyl alcohol.) The neutral red content of the egg sample was then determined by comparing (in a Duboscq colorimeter) the intensity of the extract with a standard neutral red solution. The neutral red content per unit volume of eggs was then calculated. (Maximum error; 3 per cent.)

Curves were plotted indicating the neutral red content of the eggs after various periods of time in the stain. Curves of similar shape were obtained for *Chaetopterus* and *Nereis* eggs. The maximum staining rate occurs during the first 20 minutes. Thereafter, the velocity of the process decreases until after 60–80 minutes an equilibrium is reached and no further change in neutral red content occurs.

At concentrations below .02 mgm./ml. the staining rate is proportional to the concentration of neutral red in the suspension. The stain content of the eggs at equilibrium is also proportional to the concentration of neutral red.

The initial staining velocity and the quantity of neutral red bound at equilibrium increase with temperature. Within the temperatures noted (concentration: .01 mgm./ml.) the Q_{10} obtained from the initial velocities of the staining curves of *Chaetopterus* eggs was 4.4.

The adaptation of Paramecium to sea water. John A. Frisch, S.J.

In cultures of hay and wheat infusions, in which the concentration of sea water was gradually increased by evaporation, many individuals survived and divided until a concentration between 35 and 40 per cent was reached; all died before a concentration of 45 per cent was reached. Daily observations of a culture in which the concentration of sea water increased to 40 per cent in 20 days showed that the average rate of pulsation and the average rate of feeding were lower day for day than in fresh water cultures; that both rates varied from day to day as in fresh water cultures, and that the rate of pulsation varied with the rate of feeding, increasing or decreasing from day to day, as the rate of feeding increased or decreased, just as in fresh water cultures. As the salt concentration increased the animals decreased in length and volume and became emaciated. Addition of nutrient medium or of bacteria to the cultures always resulted in an increase in the number and in the volume of the individuals, and in the rate of feeding and the rate of pulsation, except when the concentration of sea water had reached 40 per cent. The data indicate that the decrease in the rate of pulsation is not due to the increase in osmotic pressure of the medium, but to the decrease in the rate of feeding; that the decrease in the rate of feeding is due to a shortage of bacteria in the higher salt concentrations; that death is due to an increase in the viscosity of the protoplasm and to other toxic effects of the salts taken in by the cytostome, which result in the vacuolization of the protoplasm.

The water and fat content of skeletal muscle in marine fishes. Charlotte Haywood and Abby Turner.

In the course of a study of water balance in fishes of different life habits and phylogenetic relationships a series of determinations was made of both the water and fat content of skeletal muscle. This work was done in part at Bergens Museum Biologiske Stasjon, Herdla, Norway, and in part at the Marine Biological Laboratory, Woods Hole.

	No. of Individuals	Percentage of Fats	Percentage of Water in	
			Whole Muscle	Fat-free Muscle
Elasmobranchs:				
Mustelus canis	4	Trace	78.4	78.4
*Pristiurus catulus**	4	—	80.0	—
Galeus glaucus	1	Trace	81.0	81.0
Carcharias taurus	2	0	77.4	77.4
*Squalus acanthias**	1	—	78.4	—
*Squalus acanthias**	7	5.4	75.0	79.4
*Spinax niger**	4	—	80.6	—
Raja diaphanes	1	0	75.1	75.1
Raja stabuliforis	2	0	76.5	76.5
*Raja fallonica**	1	—	79.0	—
*Raja oxyrhynchus**	3	—	81.1	—
Narcacion nobilianus	3	0.4	83.9	84.2
Dasybatus marinus	2	Trace	77.9	77.9
*Chimaera monstrosa**	3	—	81.3	—
Teleosts:				
*Anguilla vulgaris**	3	—	66.4	—
Anguilla rostrata	1	4.7	73.3	77.0
*Scomber scombrus**	6	—	66.5	—
*Scomber scombrus**	8	5.4	70.5	74.5
Centropristes striatus	11	1.0	78.7	79.5
Cynoscion regalis	2	3.1	76.7	79.2
Tautoga onitis	4	Trace	79.8	79.8
*Cyclopterus lumpus**	1	—	72.2	—
Prionotus strigatus	2	0	79.9	79.9
Echeneis naucrates	1	3.7	73.1	76.2
Opsanus tau	2	Trace	80.2	80.2
*Anarhichas lupus**	6	—	84.0	—
*Gadus callarias**	8	—	80.3	—
*Gadus callarias**	6	0	81.7	81.7
*Gadus pollachius**	7	—	79.4	—
*Brosmius brosme**	6	—	80.0	—
*Molva molva**	2	—	80.3	—
*Lophius piscatorius**	5	—	84.3	—
*Lophius piscatorius**	2	0	87.1	87.1

* Determinations made in Norway.

Reactions to light of different intensities in Dolichoglossus kowalevskyi. Walter N. Hess.

For studying the sensitivity of *Dolichoglossus* to different light intensities a 100-watt Tungsten bulb, supplied with neutral tint Wratten filters, was used and light of 115, 11.5, 1.15, and .115 m.c was thus obtained. For studying the relative photosensitivity of different regions of the body a fused quartz rod was suspended so that its base was illuminated by an arc lamp. The distal end, which was used in testing photosensitivity of small areas, was drawn out into a very small blunt point.

The animals reacted negatively to ordinary intensities of light but at .115 m.c. a rather large majority of positive responses occurred. By means of the pin-point light it was shown that the animal is photosensitive over its entire body though certain regions are more light-sensitive than others. The tip of the proboscis is the most sensitive to light. In general, the dorsal and lateral body surfaces are more photosensitive than the ventral surface.

Mucous cells occur on the abdomen in patches interspersed with non-mucous cells. Very little response was obtained by illuminating the areas of mucous cells especially on the ventral surface, yet if the areas of non-mucous cells were illuminated the animals responded quickly.

These results show that the photoreceptor cells must be widely distributed and that they must be more numerous or more specialized in certain regions than in others. Cells of a certain type which correspond in their distribution to the relative photosensitivity of the animal have been identified. In keeping with the early chordate characteristics of this animal these cells resemble in their general morphology the retinal cells of vertebrates.

The hatching of the squid. Hope Hibbard.

The hatching gland of *Loligo pealii* is the so-called Hoyle's organ, a Y- or T-shaped gland which lies on the dorsal posterior surface of the larva, the arms of the gland extending out on the fins. It appears very early (about stage 12 of Naef in *L. vulgaris*), gradually matures, and disappears entirely shortly after the larva hatches. At the height of its development the gland protrudes slightly. A similar hatching gland has been described by Wintrebert and by Jung in several other cephalopods found in Europe. Staining the eggs or young embryos, even very heavily, with neutral red does not impede their normal development, and the organ in question can be very readily observed since it does not stain and appears as a white streak against a reddened mantle.

In early development the larva rotates inside its shell by ciliary action, pushing the posterior end up until gravity causes it to lose balance, and fall to the lower side of the shell whence rotation recommences. But as hatching approaches the animal adheres very firmly to the shell as if stuck by a secretion, in a disc-shaped area around the hatching gland. The head is directed downward, and rhythmic contractions of the free part of the mantle are constant. The gland itself appears to wriggle and squirm from time to time, due to underlying muscle cells, pushing ever closer to the shell. In some cases the weak place bulges considerably before giving way. Finally the shell appears to dissolve away and the animal swims out backward through a neat round hole. There is no tearing, and after the larva has emerged the round hole remains in the empty shell.

The cells of the gland are very long, slender ones with the nucleus at the proximal end deeply embedded in the mantle. The secretion is conspicuously granular and resembles well-fixed zymogen granules in a pancreas cell. Pressure on the cells forces the granules out, and they maintain their individuality outside. After hatching, the cells remain for a few hours, but the distinctness of the unsecreted granules disappears, the material becomes more fluid and runs together. Further cytological examination of the evolution of these cells is under way.

The vacuole system of the marine Amoeba, Flabellula mira. D. L. Hopkins.

In this study the vacuole system was observed when the amoebae were under normal conditions; slightly abnormal conditions; unstained; stained with the vital dyes, neutral red, Nile blue sulfate and Janus green B; and after impregnation with osmium tetroxide.

Mitochondria which stain with Janus green B are not present in normal fully active amoebae, but appear and may be stained with Janus green B when the amoebae are placed under slightly abnormal conditions. The mitochondrial substance in fully active amoebae appears to be contained in very small vacuoles. These vacuoles arise de novo from the protoplasm, grow, fuse with each other and engulf food. The food vacuoles and the vacuoles containing no food fuse with each other, forming larger and larger vacuoles, until finally a large fusion vacuole, the *cloacal vacuole,* is formed. The fluid and solid contents of this cloacal vacuole after a period of digestion are voided to the outside.

Nile blue sulfate stains all these vacuoles blue from the time of their origin until after their entrance into the cloacal vacuole where the blue fades out, in some cases becoming clear of color directly, while in others it becomes pink or red and then clear. This indicates that fatty acids are present in all vacuoles, and perhaps some neutral fat. Both of these types of substances disappear from the vacuoles before the residue is voided.

The substance stained by neutral red is present in all the smaller vacuoles but disappears entirely before they enter into the cloacal vacuole.

Neutral red staining, and osmium tetroxide impregnation of amoebae previously treated with neutral red show that the smaller vacuoles and the cloacal vacuoles are in a more highly oxidized, or in other words, in a less reduced condition than are the vacuoles of intermediate size.

Effect of electrical shocks upon the division rate of Stylonychia pustulata as measured by the interdivisional period. Lois Hutchings.

The normal interdivisional period of *Stylonychia pustulata* in summer was found to be six to ten hours. Most frequently the control animals underwent division once every eight hours. Every animal used in these experiments came from one protozoön. Isolation dishes were used. The culture medium was filtered hay-tea in which mass cultures had lived previously for five to twenty days, average pH 7.4. A piece of dry outmeal, one-millimeter square, was placed in each dish when the medium was changed each day.

Two new dry-cell batteries were connected in series to the primary circuit of an inductorium, Harvard type. The secondary coil was kept at the eight-centimeter mark. The current which flowed through the secondary circuit had a non-smooth alternation of 106 times a second, known as tetanizing current. A foot switch closed the circuit at will. From the secondary circuit were two insulated copper wires to whose tips were soldered one-inch lengths of No. 30 platinum wire. Each point electrode was so manipulated by hand that a constant distance of twice the body-length between it and the animal was maintained. Even when the protozoön moved it was usually possible to keep the interelectrodal distance constant.

Each *Stylonychia* received treatment hourly. For several reasons physiological indices of sufficient treatment were adopted. In order of importance these were: degree of swelling, loss of lateral orientation to the current, and rapid spinning. The length of treatment varied from 10–75 seconds.

Disregarding the five cases in which the treated *Stylonychia* had the same length of interdivisional period as the control animals, the results may be simply stated. Fifteen treated animals underwent an average shortening of the interdivisional period by 2 hours, but 102 animals had an average lengthening of the interdivisional period by 6¾ hours. The range of shortening was ½-3½ hours and the range of lengthening was ½–23 hours. In other words, although in 13 per cent of the cases application of electrical shocks caused an apparent acceleration of the division rate, in far the greater number of cases, 87 per cent, such shocks caused a slowing down of the division rate.

Further comparative studies on the permeability of the erythrocyte. M. H. Jacobs and H. N. Glassman.

In earlier papers (*Proc. Am. Philos. Soc. 70,* 363, 1931; *Jour. Cell. Compar. Physiol. 7,* 197, 1935) certain characteristic differences in the permeability of the erythrocytes of different species of vertebrates to dissolved substances were reported. The results previously obtained by means of the hemolysis method were not very satisfactory in the case of many species having nucleated erythrocytes because of the failure of suspensions of the latter to become completely transparent on hemolysis. By taking advantage of the fact that this difficulty is largely removed by the addition to the hemolytic solutions of 0.001 M $NaHCO_3$, without greatly altering the time of hemolysis in the case of most of the species studied, further data have been obtained on the permeability to ethylene glycol, glycerol, urea, and thiourea, of the erythrocytes of 9 species of elosmobranchs, 14 of teleosts, 2 of frogs, 6 of turtles, 4 of snakes and 4 of birds. In addition to various highly characteristic specific differences the following general peculiarities of the larger groups—subject to some exceptions which cannot here be mentioned—are of interest. *Fishes:* permeability to ethylene glycol greatest; that to urea and glycerol highly variable from species to species; permeability to thiourea usually greater than that to urea. *Amphibia:* (data as yet too scanty to permit generalizations). *Birds* (gull, tern, English sparrow, pigeon): permeability to ethylene glycol and glycerol both very great and nearly equal; that to thiourea much less and to urea least of all. Earlier experiments showed similar conditions in the starling but not in the duck and the chicken. *Reptiles:* permeability to urea relatively great, followed by that to ethylene glycol and at a much greater distance by that to thiourea. Permeability to glycerol slight as compared with that to the other substances. *Mammals:* (for comparison) permeability to urea extremely great; that to ethylene glycol much less and to thiourea still less; permeability to glycerol in some species greater and in some species less than that to thiourea.

The attenuation of toxins by interfacial adsorption. J. M. Johlin.

In view of the fact that some toxins, upon standing, are changed into toxoids, the writer thought it likely that similar attenuations might be brought about more speedily by methods which induce interfacial adsorption, and has applied such a method to the attenuation of ricin, tetanus toxin and snake venom. This speedy attenuation may be regarded as the result of the catalytic influence of the interface in bringing about an increased surface concentration of properly oriented molecules of the toxin at the interface. Adsorption was brought about by emulsification of the toxin with a volatile inert liquid such as ether or chloroform which could be easily removed by evaporation under reduced pressure afterwards. Ricin, thus attenuated, was found to be ten thousand times less active when injected intracutaneously into rabbits. One thousand M.L.D.'s of tetanus toxin, when attenuated, could be injected subcutaneously into mice repeatedly at two-day intervals without killing them or causing any apparent prolonged discomfort. Multiple lethal doses of mocassin and rattlesnake venom could also be injected into mice without causing death or producing the usual signs of damage caused by the untreated venoms. In such immunization experiments with rabbits as have been carried out it was found that the attenuated toxin had retained its antigenic property and could be injected daily in large amounts without producing visible symptoms of any kind.

The action of acetylcholine on the skeletal muscle fibers of the frog. Elsa M. Keil and F. J. M. Sichel.

In a previous report by the present authors (*Biol. Bull.* 71: 402, 1936) it was shown that the application or injection of very small amounts ($5000 \mu^3$) of acetylcholine in concentrations ranging from 1 in 10^3 to 1 in 10^{11} had no effect on the isolated single fiber (adductor muscle of frog's leg, *Rana pipiens*), beyond the effects attributable to the medium in which the acetylcholine was dissolved. Recent experiments with acetylcholine on single muscle fibers isolated from the sartorius of the frog confirm this. However, if the sartorius be dissected from the frog, and even if both the tibial and pelvic ends be cut across, then acetylcholine will cause a propagated twitch when applied as a small droplet with a micropipette to the dorsal surface of the muscle in the region of the nerve twigs. This twitch is noticed in only those muscle fiber bundles which are in the vicinity of the point of application of the acetylcholine. If either the pelvic or tibial half of the sartorius is split lengthwise into two parts, one containing about twenty fibers, the other the rest of the fibers, then acetylcholine applied to the surface of either of these bundles also causes a similar propagated twitch response. If the smaller bundle of fibers is progressively split into still smaller groups, a degree of subdivision will eventually be reached such that even dilute acetylcholine will no longer evoke twitches in fibers still irritable to electrical stimulation. This apparently occurs when the subdivision has interfered with the nerve supply, the fine nerve twigs being injured by the process of dissection.

We conclude that these experiments offer further evidence in support of our previous statement that the action of acetylcholine is not upon the contractile mechanism of the muscle directly. We further conclude that acetylcholine evokes twitches in the sartorius muscle of the frog only when the terminal nerve supply or possibly some junctional tissue or receptor is intact. These conclusions are in accord with Garrey's experiments on turtle and *Limulus* heart, and with Armstrong's experiments on embryonic *Fundulus* heart.

The effect of pituitary on nuclear changes in the egg of the frog. John A. Moore.

In an effort to separate the processes of maturation and ovulation in the frog, *Rana pipiens,* mature females were given homoplastic anterior lobe injections during the middle of the summer, at which time it has not been possible to stimulate the release of eggs. Parts of the ovary were removed before and after injection so the same animal serves as a control and as an experimental. It was found that by thirty to forty hours after injection (at 25–27° C.) the germinal vesicle had moved from its position in the center of the egg to the surface at the animal pole. This movement is found only in the large eggs with evenly distributed yolk. By one hundred and twenty hours the eggs are degenerating and have been heavily invaded by phagocytes. This latter condition is similar to that found by Miss King in the few mature eggs that remained in the ovary of the toad after the breeding season.

The effect of urea upon the surface of unfertilized Arbacia punctulata eggs. Floyd Moser.

Following the procedure of Moore (*Protoplasma,* 1930) *Arbacia punctulata* eggs were treated with molar urea solutions. After a total exposure of no more than two minutes, the eggs were transferred to sea water. Some of them were then inseminated and others served as uninseminated controls.

Within three or four hours irregularly cleaving eggs (two to eight cells) may be found among both the treated inseminated eggs and the treated uninseminated eggs. At the same time apparently normal blastulae with membranes perhaps a little thinner than normal may be found among the treated inseminated eggs, but not among the uninseminated eggs. In some cases it is possible to demonstrate very thin and but slightly elevated membranes in these irregularly cleaving eggs.

If a small drop of sea water containing centrifuged or uncentrifuged eggs be placed on a slide and a larger drop of molar urea solution be caused to flow into the drop of eggs while the latter are being observed under the high power of the microscope, a breakdown of cortical granules occurs. Immediately following this cortical response membrane elevation occurs with the formation of a very wide perivitelline space. Within a few seconds the elevated membrane begins to recede toward the egg surface, becoming thinner as it moves, until finally, in some cases, no vestige of the membrane remains. The cortical response secured in these urea-treated eggs is essentially like that obtained upon stimulation with sperm cells or with agents which induce parthenogenesis such as saponin. Similar cortical responses are secured in molar thiourea and molar glycerine solutions.

It is evident, therefore, that these non-electrolyte solutions do not prevent membrane elevation in *Arbacia punctulata* eggs (either irreversibly or otherwise), but may actually stimulate the egg to cortical response and subsequent membrane elevation. Indeed, delayed and irregular cleavage often follows the cortical response after treatment with these agents.

The cortical response of Arbacia punctulata eggs to direct current.
Floyd Moser.

In a previous abstract (*Biological Bulletin,* 1935) it was noted that a layer of granules located in the cortex of the unfertilized *Arbacia* egg breaks down when the egg is subjected to a number of different stimulating agents, as well as to normal insemination. The present experiments with direct current extend the list of stimulating agents reported earlier.

A non-polarizable system of Cu-$CuSO_4$ electrodes with agar bridges was used. The eggs were placed in small glass tubes both ends of which were plugged with 2 per cent agar made up in sea water. The current intensity varied from 1.5 to 20.0 milliamperes.

As soon as the circuit is closed the eggs begin to move toward the anode. Within a few seconds cortical layer granules on the anodal side of the egg break down, releasing the fertilization membrane from that portion of the egg over which the response has taken place. When the current is reversed the same response takes place on the opposite side of the egg. Generally some of the cytoplasmic granules beneath the cortex also exhibit this breakdown phenomenon. Indeed, when the current flows for too long a time the cortical response is followed or accompanied by complete cytolysis.

In the uncentrifuged eggs the cortical response stops when the circuit is broken, but generally completes itself in wave-like fashion in the centrifuged egg. The so-called fifth layer usually breaks down in the centrifuged eggs.

If the eggs are treated with M/4 ammonium oxalate, 0.3 M potassium oxalate or 0.35 M potassium citrate there is no granule breakdown or membrane elevation upon exposure to direct current.

The observations here recorded are in general agreement with the point of view developed by Heilbrunn and his students in their studies of stimulation.

The histology of the retractor muscles of Thyone briareus Lesueur. Magnus Olson.

A decided gap exists in our knowledge of the comparative histology of invertebrate muscle. This fact has made desirable a more comprehensive study of invertebrate muscles particularly since a considerable number of these have been employed in recent physiological research.

The only extensive paper on the histology of holothurian muscle (Hall, 1927 on *Cucumaria*) misinterprets their essential structure and has been responsible for some erroneous conclusions by workers in muscle physiology.

The present study has shown that the retractor muscles of *Thyone* consist of enormously long narrow unstriated fibers imbedded in a connective tissue network. The fibers occur in bundles of 2–15 and in each bundle are arranged circularly in a single layer. Each bundle is surrounded by a comparatively dense layer of connective tissue which penetrates only sparingly into the interior of the bundle. Hall interprets the bundles of fibers as being single cells each of which contains many enormously large fibrils. That these so-called fibrils of Hall are actually the muscle fibers can be determined readily not only by the presence of connective tissue within the fiber bundles but also by a study of the arrangement of fiber nuclei. Critical examination of this arrangement reveals a peculiar picture. In the contracted fiber the nucleus lies on the outside of the fiber and may be connected to it only by a narrow protoplasmic strand. In the extended condition of the fiber the nucleus is elongated in the axis of the fiber and lies closely pressed against it. Occasionally nuclei are found directly within the fibers. As far as it has been possible to determine, the fibers appear to be uninucleate. Connective tissue nuclei occur not infrequently within the bundles of fibers.

The fibers are extremely extensible. The muscles may vary in length from about 5 mm. in the contracted condition to 5 or 6 cm. in normal extension or after anaesthetization with magnesium chloride. The fiber diameter varies from 5–10 mu in a contracted condition to 2–4 mu in an extended condition.

From teased preparations it may be seen that although the fibers are extremely long, they do not extend the full length of the muscle. The terminal portions of the fibers are long and tapering. The fibers are spherical or hemispherical in cross-section in a contracted condition, laterally compressed in an extended condition. The connective tissue fibers appear to run at right angles to the muscle fibers and form a dense network at the periphery of the muscle.

Strength-duration curves of nerve fibers in the squid. C. Ladd Prosser and A. H. Chambers, Jr.

Giant nerve fibers and fin nerve fibers of the squid were stimulated by condenser discharges through calomel electrodes and strength-duration curves were obtained. Excitation constants (Hill's characteristic time, k) range from .33 to .60 milliseconds and average .47 milliseconds for the isolated giant fibers. The time constants are essentially the same (characteristic time averages .42 milliseconds) for the giant fiber intact in the stellar nerve as for the isolated fiber, but the whole strength-duration curve is shifted upward with the intact nerve. Hence the effect of other fibers in a nerve trunk upon the excitation of one fiber is to serve as a shunt for the stimulating current.

Decreasing the electrode separation from 12 to 3 mm. shifts the strength-duration curve diagonally upward and to the left, with the result that the time constants are shorter at the smaller separation. As the preparation deteriorates the threshold rises and the characteristic times become shorter, i.e. the change in the strength-duration curve is similar to the change resulting from shortening interelectrode distance. With electrodes 7 mm. in diameter the time constants are approximately 1.5 times as long as with electrodes less than 0.6 mm.

The diameters of the giant nerve fibers are approximately 100 times those of the fin nerve fibers. The fin nerve fibers have characteristic times approximately twice as long as the giant fibers (.88 millisecond with electrode separation of 12–14 mm.).

New structures induced by implants of adult nerve cord in the polychaete, Clymenella torquata. Leonard P. Sayles.

For several summers a study has been made in which a piece of adult nerve cord, removed from one *Clymenella,* was transplanted into another. All implants were of one to two segments in length. They were placed in the body wall in a dorso-lateral region to minimize the chance of injuring the nerve cord of the host. No bud has been formed in any case in which the nerve cord slipped into the coelom free from the body wall.

To date 63 buds have been induced by this type of implant. These include 12 heads, 29 tails, 2 double buds each consisting of a head and a tail, and 20 irregular or weakly developed buds. No head buds have appeared posterior to the tenth segment of the host. One of the double structures was formed at the fourth, the other at the eighth.

Both the source of the implant and the region of implantation in the host seem to influence the type of bud to be formed. Many more cases must be secured before any conclusions can be drawn concerning this point. The possibility that orientation of the implant may also be a factor has not been fully eliminated.

Cytoplasmic division in type B of the giant amoeba Chaos chaos Linnaeus. A. A. Schaeffer.

Although type A and type B of *Chaos chaos* are very closely similar, morphologically, to the common laboratory amoeba, *Chaos diffluens,* there are certain characteristics which set them apart very definitely. One of these characteristics is the striking phenomenon of frequent division into three daughters instead of two, which occurs in both types of *chaos.* During division the amoebas of type A round up into a mulberry-like shape, while those of type B, which are also berry-shaped at first, soon flatten out somewhat, so that one cen predict to a certain extent the number of daughters about to be produced, by counting the well-defined lobes of the flattened-out amoeba. The number of daughters may be 0, 2, 3, 4, 5, or 6, and the number of lobes formed before separation is well correlated with the number of daughters produced. The amount of undigested food in the amoeba influences the number of daughters to be formed, to a markedly greater extent than it influences the number of lobes formed during division. In both cases the number is reduced by large quantities of undigested food.

In type A, more divisions result in 3 daughters than in all the other classes put together, while in type B, in a culture with food in excess of needs, as many amoebas divide into 2 as into 3. But the number of amoebas showing 3 lobes prior to division, under these cultural conditions, is much greater than those with 0, 2 and 4 lobes combined. The average variation in size between twins from a 3-lobed parent is also greater than that between twins or triplets whose parents showed 2 and 3 lobes respectively before separation of daughters. More sextuplets occur than would be expected on pure chance, although not enough data are at hand to warrant the conclusion that the curve of frequency rises again to some degree at 6 daughters.

Something akin to a trigonal field of force in an apparently turbulent medium exists here which stands in striking contrast to the common halving type of cytoplasmic division.

The sense of taste in the free fin rays of the sea robin (Prionotus). Ernst Scharrer.

Experiments were carried on to show that the free fin rays of the sea robin are chemoreceptors as well as organs of locomotion. Blinded sea robins react after 3–4 weeks of acclimatisation in the aquarium with snapping reactions when the juice of a spider crab or of a clam is gently poured on the free fin rays by means of a pipette. A water current caused by a pipette never is followed by a reaction. The behavior of the sea robin is therefore identical with that of the Mediterranean genus *Trigla* (Scharrer, 1935) and it might be concluded that all Triglidae use the three free rays of the pectoral fins as chemoreceptors in search for food.

Calcium and magnesium in relation to longevity of Mactra, Nereis and Hydroides egg cells. Victor Schechter.

Experiments on the relation of calcium to longevity of unfertilized *Arbacia* egg cells (*Biol. Bull.,* **72**: 366) were extended to the cells of the mollusk, *Mactra,* and to those of *Nereis* and *Hydroides.* The *Mactra* egg, which in change in rate of hypotonic cytolysis with age (*Biol. Bull.,* **71**: 410) follows the *Arbacia* egg closely, also exhibits prolongation of life in low calcium most clearly. This action, as in *Arbacia,* seems to be one rather specific to calcium as a decrease in magnesium alone does not produce it nor does lowering of both magnesium and calcium have an added effect. Increase of magnesium above the normal concentration in sea water did result in an additive effect on longevity.

The two phenomena need not, however, be related. The first sign of deterioration in the *Mactra* egg is usually the breakdown of the germinal vesicle. Insemination also causes germinal vesicle breakdown. In eggs which cannot be activated by sperm, cytoplasmic cytolysis may occur with age while the germinal vesicle is still intact. With such eggs, increasing the magnesium concentration retarded cytoplasmic disintegration; and the eggs then lived longest if calcium was low.

Therefore, in so far as the phenomena of aging may be partitioned between the various cellular components, the effect of low calcium is tentatively regarded as one which retards deteriorative changes in the nucleus, whereas the action of high magnesium may be primarily in the nature of an anaesthesia. Magnesium may act chiefly on the cell membrane since with increased magnesium the egg cells retain their spherical shape beyond the time when controls appear plasmolyzed.

Preliminary experiments with *Hydroides* eggs show prolongation with low calcium.

In one-sixth the normal calcium concentration the reaction of *Nereis* eggs was variable. This is possibly due to egg condition and it may be that with different calcium and magnesium concentrations prolongation of life in these cells will also be obtained consistently.

A convenient method for the measurement of nerve respiration. Francis O. Schmitt and Otto H. Schmitt.

For the determination of the effect of electrical stimulation on the oxygen consumption of single nerves it is desirable that readings be made over short intervals of time (every 2–3 minutes) and that the variations between individual readings be small (ca 2–5 per cent). Even with differential volumeters of small volume this requires that temperature fluctuations between the two vessels be reduced to a minimum. This may be accomplished without the use of a precision thermostat by immersing the vessel in a mercury bath, the latter in turn being enclosed in a well insulated box. The mercury is contained in a copper box lined inside and outside with insulating material and provided with a glass window which permits following the movement of the index droplet with a traveling microscope, the capillary being only partially immersed in mercury and bent so that the vessels are submerged well below the surface of the mercury. Wires from the electrodes are brought out through rubber tubes. The capillary is illuminated by filtered light brought in by a glass rod fastened on the carriage of the travelling microscope. The copper box is surrounded by kapok or eiderdown contained in a well-insulated box which also carries the traveling microscope. The entire unit is easily portable and, at least for work at room temperature, the method has been found quite as satisfactory as the conventional method which requires a precision thermostat of special design.

Temporal relations in the excitation of the isolated muscle fiber. F. J. M. Sichel and C. Ladd Prosser.

The possibility of spatial summation in the excitation of the isolated muscle fiber has been reported previously (Sichel and Prosser, *Biol. Bull.,* **69**: 343, 1935). In the present case the excitatory effects of two stimuli separated by a varying interval have been studied. The stimuli were obtained from two condensers, each controlled by a gas triode, discharging into a common resistance. The interval between the two shocks was varied by a Lucas' spring rheotome in the grid circuits of the gas triodes. The time-constant for the discharge of each condenser was 1 millisecond.

The fiber (adductor longus, *Rana catesbiana*) was isolated and mounted in a manner previously described (Sichel, *Jour. Cell. Compar. Physiol.* **5**: 21, 1934) and the isometric tension recorded by a micro-lever (Brown and Sichel, *Jour. Cell. Compar. Physiol.* **8**: 315, 1936). The electrodes were silver-silver chloride, and were sufficiently large to make the spreading of the field at the ends of the fiber unimportant.

The tension developed falls continuously as the interval between the shocks increases, rapidly at first, then more and more slowly toward a steady value reached when the interval between shocks is about 20 milliseconds. The tension is then one-third to three-quarters of the tension developed when the stimuli are simultaneous.

If a similar experiment is done on the whole sartorius using maximal stimuli, as the interval between stimuli increases the tension at first remains constant, this being related to the absolute refractory period, then increases rather abruptly to a new level. With submaximal stimuli there is an initial fall to the constant level followed by a subsequent rise.

The absence in the case of the isolated fibers of the low constant level of tension for short intervals, and the smooth nature of the tension-interval curve we attribute to the absence of an absolute refractory period in the excitation of these fibers.

Electrolytes in Phascolosoma muscle. H. Burr Steinbach.

The retractor muscles of the marine annelid *Phascolosoma gouldi* consist of closely packed long smooth muscle fibers. Muscles freshly excised from the animal contain the following average amounts of inorganic elements, expressed as milliequivalents per hundred grams wet weight: Na 10.4, Cl 9.1, K 10.6, Ca 0.85. The body fluid surrounding the muscles contains approximately: Na 38, Cl 43, K 3.5, Ca 2.0. On immersion for two hours or more in sea water, the tissues gain weight by 11 per cent, chloride increases by 16 per cent, Na by 14 per cent, Ca by 13 per cent while 11 per cent of the K is lost. These changes can be partly accounted for by assuming an extra-cellular chloride space which alone is involved in the tissue swelling and the substitution of sea water for body fluid. Both Cl and Ca increase somewhat more than would be expected on this simple hypothesis.

The Na increase is less than would be expected while a large part of the K loss must involve the fibers themselves.

Muscles were soaked for two hours or more in artificial sea water containing known concentrations of K or Ca, or in sea water diluted with isotonic sucrose solution.

Na, Cl and Ca contents of the muscle bear a simple linear relationship to the concentrations of these ions in the immersion fluid. All of the Na and Cl appears perfectly free and diffusible, the slope of the curves Na-tissue/Na-fluid and Cl-tissue/Cl-fluid indicating about 30 per cent chloride (extra cellular) space at all concentrations. About half of the initial Ca content does not diffuse into Ca-free sea water or isotonic sucrose, but using that figure as zero concentration of Ca in the muscle, the curve for Ca-tissue/Ca-fluid is linear and has a slope indicating about 40 per cent diffusion space. Eighty per cent of the K does not diffuse into a K-free medium. As the K content of the fluid is increased the K of the muscle increases, rapidly at first then more slowly until finally a linear relationship is shown between K-tissue and K-fluid at concentrations between 0.05 M and 0.25 M K in the fluid.

Leaf development and vegetative propagation in Polystichum plaschnickianum. Harry N. Stoudt.

Material for this work was collected by the writer in the rain forests along the Vinegar Hill Trail, Jamaica, B. W. I. in the summer of 1936 and the investigation was undertaken at the suggestion of the late Professor Duncan S. Johnson.

Leaves of this plant are produced from segments of the apical cell of the rhizome. The leaf grows, apparently, by means of a bifacial initial which persists in the mature leaf. This cell continues to cut off segments to form the first embryonic leaf of the plantlet at the apex of the leaf. An apical cell is cut out from a marginal cell of one of the last segments of the parent leaf to form the shoot of the plantlet. Later leaves are formed from segments cut off from this cell. A meristematic cushion forms at the apex of the parent leaf and adventitious roots arise endogenously in this region and grow by means of a tetrahedral initial. By this time the roots penetrate the moist soil and the plantlet matures. Simultaneous with plantlet development the parent leaf develops wings that encircle the plantlet. Further investigation is needed to understand the origin and development of these wings. When several leaves and roots have formed the plantlet becomes independent and this region of the parent leaf decays. The remaining portion of the mother leaf persists to carry on spore formation.

Morphological and experimental cytology of lobster spermatozoa. (Preliminary note.) T. Terni.

In Woods Hole I have begun to microdissect the spermatozoa of decapods, most appropriate material because of its immobility and large size. I refer here only to my preliminary results.

1. The three long rays of the head have a permanent shape which is slightly curved. They are elastic, and if deformed by the needles, they immediately resume their original form. The rays are, on the other hand, neither plastic nor extensible if pulled with the needle. The mechanical stimulation does not induce visible contraction of the ray-like filament. Movements of these, if really existing, are in all cases so slow that only the microcinematographical technique will reveal them.

2. If with the tip of the needle one exerts considerable pressure, although not sufficient to injure, on the anterior part of the spermatozoa, there always appears the phenomenon of the so-called "explosion" (Koltzoff). The process seems to be irreversible; I have followed for 20 hours the fate of the exploded spermatozoa without changes. In order that the explosion should take place after pricking, it is necessary that the spermatozoa be freshly removed from the spermaduct.

3. In the posterior part of the small tube contained in the chitin capsule of the tail there exists before the explosion material strongly acidophilous. After the explosion the capsule and its content become suddenly basophilous while the nuclear chromatin loses its strong basophily, perhaps because it diffuses in the capsule.

4. With microdissection it is easy to follow the very rapid process of invagination of the anterior part of the spermatozoön into the capsule, which simultaneously becomes swollen. The examination of good preparations (ac. osmic-iron hemat.) shows the fact that the basis of the cephalic rays are pulled into the tail portion like an umbrella that closes itself into a sheath. Simultaneously from the posterior pore of the tube there flows out a basophilous body, perhaps the anterior centrosome.

Observations on arterial pressure in marine fishes. Abby H. Turner and Charlotte Haywood.

In an effort to determine representative values for the arterial pressure supplying the organs of fishes the anterior mesenteric artery was employed for cannulation. Pressures were measured in centimeters of isotonic saline, using a manometer. Respiration was well maintained by a stream of water over the gills, but obvious disadvantages in the method are that the fish is not entirely submerged in water and that the body cavity must be opened.

A number of the determinations were made in the summer of 1936 at Bergens Museum Biologiske Stasjon, Herdla, Norway.

	No. of Individuals	Range of Values in cm. Saline	Average Values in cm. Saline
Elasmobranchs:			
Mustelus canis	4	11. –25.5	16.7
Carcharias taurus	2	16. –19.	17.5
Squalus acanthias	7	8.5–18.8	15.1
Raja diaphanes	1		22.
Raja stabuliforis	2	12.5–21.5	17.
Raja oxyrhynchus	1		8.3
Narcacion nobilianus	3	Extremely low	
Dasybatus marinus	2	9. –10.1	9.5
Teleosts:			
Centropristes striatus	1		37.
Anarhichas lupus	4	9.7–15.	11.3
Gadus callarias	12	10.9–43.	27.7
Gadus pollachius	2	12.2; 12.2	12.2
Lophius piscatorius	6	9. –23.7	16.7

Respiratory rate and length of fertilizable life of unfertilized Arbacia eggs under sterile and non-sterile conditions. Albert Tyler, Nelda Ricci and N. H. Horowitz.

The respiratory rate of unfertilized eggs of marine animals does not remain constant with time, but sooner or later shows a marked rise. This rise occurs at the time when the eggs lose their fertilizability (Tyler and Humason, 1937). Measurements of the respiration of *Arbacia* eggs in 2 per cent alcohol or other agents that are known to prolong the fertilizable life (Whitaker, 1936) show that the rise is correspondingly delayed, while the initial absolute rate is unaffected.

By plating out for marine bacteria from the respiration vessels, it is found that the bacteria increase as the rate of respiration rises both in sea water and in alcohol. When sterile or almost sterile cultures of eggs are run it is seen that the respiratory rate remains low and shows even a slight drop. Such eggs have been run for four days without showing a rise in respiratory rate, whereas under ordinary conditions the rise begins at about 10 hours.

Culturing eggs under sterile conditions in sealed dishes prolongs their fertilizable life as Gorham and Tower (1902) found, some of the eggs lasting as long as 20 days. Under such conditions alcohol appears to shorten the fertilizable life. Addition of bacteria accelerates somewhat the disintegration of the eggs. Alcohol in ordinary sea water or in the presence of small amounts of bacteria considerably prolongs the life of the egg. In the presence of large quantities of bacteria, 2 per cent alcohol is toxic within a few hours, such cultures showing a drop in pH to as low as 4.60.

The results evidently mean that bacteria contribute to the destruction of the eggs only after certain disintegrative changes have occurred. The agents that prolong the life do not act bactericidally, but by retarding these initial spontaneous changes on the part of the egg.

The effect upon gastrulation and differentiation in Arbacia of $NiCl_2$, $CuCl_2$, and Na_2SiO_3 in modified artificial sea water. A. J. Waterman.

A formula for artificial sea water consisting of 390 cc. 1m NaCl, 9 cc. 1m KCl, 23.35 cc. 1m $MgCl_2$, 25.5 cc. 1m $MgSO_4$, 9.35 cc. 1m $CaCl_2$, about 0.1 gram $NaHCO_3$, additional water to make one liter (Chambers), and corrected for pH supports development of *Arbacia* from the blastula stage in a typical manner. Modifications and omissions of salt proportions have been studied in relation to effects on gastrulation and embryonic differentiation (Runnstrom, Lindahl, Dalcq). The addition of $NiCl_2$ and $CuCl_2$ to the above has been studied and comparison made with previous observations with these heavy metals in sea water. Increase, decrease or omission of Mg, SO_4, K, and Ca influence type and rate of effect of the metals. Exogastrulation occurred in some experiments and gastrulation could be temporarily inhibited. The blastula stage was used in all experiments.

Na_2SiO_3 was tested in relation to respiratory and cell division rates of the embryos in sea water and in the above modifications. In relatively large concentrations silicon is non-toxic and does not affect gastrulation. Normal cleavage rate is increased, top-swimming blastulae appear earlier, plutei mature sooner, and gastrulation may be accelerated. There is no noticeable differential acceleration of germ-layer differentiation. This has also been studied in artificial media where the salt balance has been disturbed or metallic salts added. Toxicity may be increased. Addition of silicon (Mast and Pace) to cultures lacking sulphur may or may not influence developmental rate.

Compounds of silicon are very abundant in nature and present in all waters (Reynolds, 1909). Both animals and plants use silicon and it is found in animal tissues. According to Mast and Pace, 1937, it increases rates of respiration and division in *Chilomonas* and has a catalytic action on synthesis of complex organic compounds.

Studies on living conjugants of Paramecium caudatum. Ralph Wichterman.

A new method of approach to a better understanding of the problem of sexuality in *Paramecium* is presented. By means of a precision micro-compressor, it is possible to observe in the living condition, the behavor of nuclear phenomena and establish time relationships during conjugation.

With a micro-pipette, a single pair of recently joined conjugants is placed on the glass slide of the compressor in a small drop of the culture medium and studied at 26° C. The metal top holding the glass cover-slip is screwed down while observations continue with the microscope. The animals are prevented from spiraling between the two pieces of glass and allowed to move around slowly. Adjustments within a few microns make this possible.

The divisions of the micronuclei and their behavior have been seen and photographed in the living condition. The micronucleus of each conjugant is seen to leave its place near the macronucleus and increase in size. While this enlargement takes approximately four hours, the end of the division (including the anaphase and telophase stages) is more rapid requiring only about 18 minutes. The "crescent" prophase stage and long anaphasic-telophasic separation spindle so characteristic of this division are clearly shown. The swelling at the center of the separation spindle becomes separated and is passed into the cytoplasm. Cyclosis moves it about until it ultimately degenerates. Each product of the first division enters into the second where again two long spindles in each conjugant are visible. The second division requires 50 minutes for completion from the time the first division products are formed. The anaphase and telophase stages are still more rapid, requiring only nine minutes. Degeneration of three of the four products of the second division is observed. Behavior of the pronuclei after the third division is being more carefully studied in an effort to record their movements accurately. The pronuclei of a given conjugant have been seen to fuse and form a synkaryon in the same individual. In a number of observations, definite evidence has been obtained to show that crossing-over of pronuclei does not occur. Too, no evidence has been obtained to show that crossing-over of pronuclei takes place.

In view of these observations, any conclusions, genetical or otherwise, based on the assumption that there is crossing-over of pronuclei, are open to grave question.

Photographs are being made of the living conjugants and will be included in the full paper. It is hoped also to make a motion picture of the entire process.

Conjugation in Paramecium trichium Stokes (Protozoa, Ciliata) with special reference to the nuclear phenomena. Ralph Wichterman.

A cytological study was made of conjugation in a race of *Paramecium trichium* Stokes. The preconjugants, which are smaller than the vegetative individuals, fuse along their oral grooves. The centrally located oval macronucleus undergoes complete fragmentation involving the formation of a twisted ribbon which becomes thinner and longer resulting in small irregular rod-like elements. These result in spherical bodies which disappear in the cytoplasm after exconjugant reorganization.

The deeply staining oval-shaped micronucleus divides three times. The first pregamic division results in two micronuclear products; the second division, four products, three of which degenerate. The remaining micronuclear product enters into the third division to produce the pronuclei. The developing third pregamic spindle is commonly seen to press against the opposite conjugant where a cone is clearly visible. There is strong evidence to believe that the pronuclei cross approximately in the mid-region of the conjugants. This point is being investigated further by observing living conjugants under the microcompressor. Pronuclei fuse to form a synkaryon which divides three times to produce eight products. Two of these divisions occur while the conjugants are together; the third division occurs in the exconjugant. Exconjugants with four macronuclear anlagen and a single micronucleus are commonly seen. This reduction in number from eight nuclear products of the synkaryon to five, is being studied further. Variations occur in regard to the distribution and fate of the eight nuclear products. Individuals with four macronuclear anlagen and one micronucleus divide with two anlagen going to each daughter while the micronucleus divides mitotically. In exconjugants with two anlagen and one micronucleus, a final division distributes a single macronuclear anlage to each daughter as the micronucleus again divides mitotically thus restoring the original nuclear condition to each animal.

This work confirms Diller's observations as reported in his abstract in *Anatomical Record,* 1934.

Mitotic activity of stimulated rat adrenals measured by colchicine technique. Opal M. Wolf.

In connection with a recent investigation it was noted that daily injections of an alkaline extract[2] of the anterior lobe of the pituitary into rats at 1 cc. and 2 cc. levels gave evidence of activity in the adrenals as measured by the colchicine technique. Control and injected animals were etherized the morning following the 2nd, 4th, 6th, and 10th days of injection. Nine to fifteen hours before the animals were killed, 0.3 mg. (per 100 grams of body weight) of colchicine[3] dissolved in distilled water was injected sub-cutaneously. At autopsy tissues were fixed in Bouin's, dehydrated by the aniline oil-dioxan method, cut at 10 micra and stained in Delafield's haematoxylin. Mitoses were counted with an oil immersion lens.

Preliminary observations were made of every 20th section in order to estimate the effect of colchicine on the organs. A 171 gram control male showed the following total counts:

Thyroid	157
Parathyroid	213
Adrenal	625 (highest count for controls)

An average count for the three largest sections of the adrenal, 45 in the controls, was used as a basis for comparison. Counts of both adrenals varied by 11–17 mitoses. Injected animals showed slight stimulation after 2 days, and greater stimulation after 4 days, but the differences were not marked for the two levels. The greatest stimulation occurred after 6 days of injection. One series of males weighing 170 grams gave:

Control	21
1 cc.	90
2 cc.	232 (2149—total for every 20 sections)

With one exception (126 mitoses on 2 cc. level), after 10 days injection the animals showed a count of 44. Nine to eleven hours after colchicine many of the cells were in early prophase, at 15 hours the greatest number were counted in the later stages. Most of the mitoses occurred in the outer part of the zona fasciculata, but a few cells in the zona glomerulosa and reticularis were dividing. No late stages of mitosis were observed in the medulla. In several instances very large nuclei appeared to be in early prophase.

[1] The experimental work was performed at Goucher College, the sections were cut and studied at the Marine Biological Laboratory.

[2] The growth extract was purchased from E. R. Squibb and Sons.

[3] Colchicine U.S.P. was purchased from Merck and Co. in the form of a powder.

The effects of the drug on the adrenal cells were similar to the descriptions by Ludford, Allen et al, and Nebel. Apparently normal stages of mitosis as well as stages leading to multi-nuclear cells were observed. The latter were probably caused by the varying concentration of the drug due to the rate of absorption and elimination. It was noted that the level of dosage had a toxic effect on some rats, others apparently showed no ill effects.

Induced breeding reactions in isolated male frogs, Rana pipiens Schreber.[1] Opal M. Wolf.

Adult male frogs give a characteristic call, show ridged epidermal thumb-pads with large, active mucous glands on the first finger of the fore limbs, develop amplexus, normally in the presence of the female, and shed sperms at the time of reproduction. In earlier work it was shown that the male as well as the female could be stimulated by the injection of frog anterior lobe to reproduce normally as early as the latter part of October and throughout the winter months. It seemed of interest to report experiments on individually isolated mature male *Rana pipiens.*

In the first experiments injections of frog anterior lobes from *Rana pipiens,* an extract of whole pig pituitary, a purified extract of the gonad-stimulating fraction of horse anterior lobe[2] and an extract of pregnant mare's serum, at times other than the breeding season and the summer, caused the shedding of sperm. Inspection of the cross-section of the testes showed empty seminiferous tubules and were comparable to the picture of the testes of frogs which were known to have fertilized eggs following pituitary stimulation. Following injection of the extract of pregnant mare's serum sperm were recovered as they passed from the red and swollen cloacal opening and stained preparations were made. In some cases the characteristic call or trill was heard 8 to 9 hours following the injection of as small an amount as one frog anterior lobe. The call was not heard following the injection of the extracts. The thumb-pads showed active mucous glands but the effect on the epidermal ridges was not so clearly shown because the thumb-pad of the adult male is ridged during the winter season.

Examination of the thumb-pads of adult males in the middle of July showed a smooth surface with slight indication of ridging and small mucous glands. A small piece from the thumb-pad of the experimental animals was removed as a control and the individually isolated animals were injected daily for 9 days with the anterior lobe from male *Rana catesbiana.* The injected animals gave the characteristic call of the breeding season, sperm were shed, active spermatogenesis was going on, the thumb-pads had developed ridges and the mucous glands were active. The thumb-pads of the experimental animals resembled those prepared from male *Rana clamitans* caught in the field and prepared immediately for histological examination. This animal breeds during the summer from June to the middle of August while *Rana pipiens* breeds from the first of April to the middle of May depending on the season and latitude.

It appears from an examination of the testes of the isolated experimental animals killed at varying times after prolonged pituitary treatment that the mature sperm are shed and spermatogenesis induced by frog anterior lobe and the extracts used in these experiments.

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[1] Part of the experimental work was performed at the University of Wisconsin Department of Zoology and part at the Marine Biological Laboratory, 1936.

[2] The extract of horse anterior lobe, Prephysin A, prepared by Chappel Bros., and the other extracts prepared in Dr. Hisaw's laboratory were obtained through the courtesy of Dr. Frederick L. Hisaw.

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EFFECT OF SALTS OF HEAVY METALS ON DEVELOPMENT OF THE SEA URCHIN, ARBACIA PUNCTULATA

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I

In previous experiments an examination has been made of the effects on gastrulation and embryonic differentiation in *Arbacia* of ether, changes in temperature, alcohols, X-rays, hypertonic and hypotonic sea water, and of the disturbance of the salt balance in sea water. Disturbance of the salt balance of sea water and the action of certain alkaline earth metals etc., have been tested on the sea urchin (*Paracentrotus lividus*) by Runnström (1935), Lindahl (1936), Lindahl and Stordal (1937) and others in relation to gastrulation, ectodermization and endodermization of the embryo and specifically to differentiation. References to other workers have been given in earlier papers. These studies have been concerned with the unfertilized and fertilized egg rather than with the blastula stage.

At gastrulation physical movements take place that result in an invaginated endodermal tube from which arises much of the mesodermal material. These movements may be delayed temporarily, retarded or inhibited, and they also may be influenced independently of differentiation. In the latter case an exogastrula may or may not form. What initiates the movements is another problem, but at least it has been shown that a host of factors, producing quite similar effects, can interfere with gastrulation; disturb the proportions of potential ectoderm and endoderm; and influence the differentiation of skeleton, body form, ectoderm and endoderm.

As regards embryonic differentiation, the effect has been in general very much the same for these various environmental modifications. Some types of differentiation, taking place as development proceeds, can be slowed down to a greater extent than others. Ectoderm, and to a lesser extent mesoderm, may grow and differentiate when endoderm formation is entirely inhibited. Furthermore, the ectoderm is

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the most resistant to environmental modifications. Different cultures may vary under apparently identical conditions; cultures from one female may vary from those of another; while individuals in the same culture always vary in their susceptibility. The exact condition of early development, i.e. fertilized egg to early gastrulation, seems of minor importance, since the results are somewhat similar under the conditions of the experiments.

Some metallic salts, which are a natural constituent of sea water, are more toxic to animal life than are others. On the whole, it is probable that the concentration of a particular metal changes very slightly throughout the ocean. The possible exception may be where large bodies of water empty into the ocean causing dilutions of certain constituents of sea water and concentrations of others, especially metals. What concentrations can the developmental stage of a littoral animal such as the sea urchin, *Arbacia punctulata*, withstand and what is the effect of increased concentration of different metals on such a developmental process as gastrulation? Finally, are the effects on development comparable to those secured by other experimental methods?

II

In this study $ZnCl_2$, $ZnSO_4$, $Zn(C_2H_3O_2)_2$, $FeCl_2$, $PbCl_2$, $CuCl_2$, $HgCl_2$, $AlCl_3$, $NiCl_2$ and $CdCl_2$ have been used in various concentrations. As in previous studies, the blastula stage was selected for study and exposure was made for long periods of time.

Reference to Tables I–IV will show the approximate concentrations which produced certain results. The concentrations are expressed as proportions and represent the amount of the metallic salt which was added to the sea water. No estimate was made of the normal amount of the metal in sea water. As nearly equal numbers of embryos as possible, without actually counting them, were used in each culture. By mixing the embryos secured from several females more or less similar cultures were secured. All results were based on random sampling from three places in the culture. The exposure time was varied, and in certain cases where developmental arrest had occurred without death, the culture was washed and returned to fresh sea water for recovery. In some instances samples were transferred at intervals from a lethal solution to fresh sea water to study the rate of the toxic action and the recovery ability of the embryos.

It is said that many of the heavy metal salts are precipitated by sea water. The concentrations specified in this study may represent initial rather than final concentrations and some of them may have changed in the course of the experiment. At this point it may be said that those experiments were discarded in which visible precipitation had occurred. The whole subject of heavy metal action is obscure.

This study was made at room temperature during the months of July and early August. Hence there was some variation as some of the experiments ran for several days. To avoid repetition only the results secured from the exposure to $ZnCl_2$ will be described, but comparisons with the other metals will be made. The data for the others are included in the tables.

III

A survey of the more recent literature has revealed numerous references to the effects of heavy metal salts on animal development and embryonic differentiation. The effect on gastrulation has apparently not been tested.

Hammett and Wallace (1928) found that the lead ion retarded growth, and differential development of the head and optic regions was markedly inhibited in chick embryos. Child (1929) has used $CuSO_4$ in the study of physiological gradients in the chick embryo. Féré (1893) obtained monsters following the injection of lead nitrate into developing chick embryos (see also Franke, 1936, for selenium salts).

Galtsoff (1932) has shown that marine invertebrate animals can concentrate different metallic elements in their bodies. Certain groups concentrate zinc, others copper, etc. Copper salts affect oyster larvæ by inducing attachment (see also Prytherch, 1931), and by initiating metamorphosis. Metallic silver causes sperm of the sea urchin to lose their fertilizing power, and paralyses plutei (Drzewina and Bohn, 1926).

Hoadley (1923) has studied the effects of the salts of the heavy metals on the fertilization reaction in the sea urchin, *Arbacia*. The inhibiting concentration varied for the different metals tested. Gold chloride was most toxic for membrane elevation and cleavage and cadmium or cobalt chloride least. Other salts tested included $CuCl_2$, $ZnCl_2$, $LaCl_3$, $AlCl_3$, $PtCl_4$, $PbCl_2$, $NiCl_2$ in the order of the toxicity. $HgCl_2$ differed from the others in that membranes elevated at a concentration of 1 part in 600,000, which was toxic for cleavage. Concentration of these metals varied slightly for different batches of eggs and thus showed the influence of a time factor.

$HgCl_2$ has a harmful effect on cleavage at different concentrations following very short exposures (Hoadley, 1930). It affects the cortical region resulting in membrane elevation. After longer exposures it affects the pigment which has mercury-avid properties. The pigment may be extruded and such an egg may develop if not injured. A conceivable mechanism is thus available by which the mercuric ion, which has entered the egg, may be bound and removed.

Copper salts are known to have an injurious effect on many organisms. In a very low concentration which inhibits fertilization, sperm may be still active. Inhibition is marked in a concentration as low as 1 part in 2,500,000 parts of sea water and is reversible provided the eggs are not injured too much. Copper appears to injure the vitality of eggs and acts as a slow poison (Lillie, 1921). A concentration of 1/62,500 is necessary to suppress cleavage. The effect of $HgCl_2$ is different from that of copper: initial stages of fertilization are little affected, susceptibility increases as fertilization progresses, fertilized eggs show the effect more rapidly than unfertilized eggs, and the movement of sperm is suppressed at great dilutions. It produces membrane elevation alone and favors it in fertilization. The effects are reversible if exposure is not continued too long. Lillie concludes from these studies that the effect of mercury and copper on fertilization following membrane formation may be due to enzyme poisoning. The effect on the initial stages of the fertilization reaction does not correspond so well to the enzyme analogy.

Glaser (1923) shows that in the egg of *Arbacia* copper becomes concentrated in the chorion, vitelline membrane and cortex. It is diffused throughout the cytoplasm. Copper occurs normally in egg pigment, membrane chorion and cortex associated with lipolysin. Normal eggs secrete copper compounds as well as removing copper sulphate from sea water. Parker (1924) has shown that marine animals will grow upon any heavy metal plate provided the metal does not liberate ions or soluble compounds. The effect of $CuCl_2$, $MnCl_2$, and $FeCl_3$ upon cardiac explants cultured in vitro has been studied by Hetherington and Shipp (1935). The survival time was tested. Other interesting studies could be given but the above are sufficient to indicate the type of work which has been done.

IV

$NiCl_2$.—In an experiment to test the effect of this metallic chloride upon the development and differentiation of the germ layers after their formation had been initiated, oval blastulæ to early gastrulæ were exposed to various dilutions of a 1 per cent stock solution made up in sea water. The cultures were examined after 21–42 hours of exposure and in some instances the embryos were returned to fresh sea water. $NiCl_2$ gave the best and most numerous examples of exogastrulation of all the metallic salts which were tried. Further-

more, the exogastrulæ underwent further differentiation. The lowest concentrations employed gave also marked inhibition of development.

After 21 hours exposure in a concentration of 1 part in 60,000, development had only progressed to a large triangular stage with skeletal spicules or short rods and initial flap formation. A few simple exogastrulæ with tri-radiate spicules were seen in the culture. During the same length of time the control embryos had advanced to a medium pluteus stage. A 42-hour exposure killed most of the experimental embryos although a few large blastulae and triangular stages showed slow movement.

In concentrations of 1/40,000, 1/20,000, 1/14,285, development was progressively inhibited but the number of exogastrulæ increased markedly. The embryos were all on the bottom of the culture dish. Both the evaginated and invaginated endodermal tubes failed to differentiate although some growth occurred, while the mesenchyme either formed small spicules or failed to show any skeleton-forming activity. The ectoderm was the least affected and continued growth. It acquired its characteristic appearance and the apical plate appeared. In such cases where the formation of the endoderm was entirely inhibited, large circular or oval ectoblastulæ were seen. A longer exposure killed the embryos, while those which had been transferred to fresh sea water showed increased activity but only slightly advanced development, especially in the case of the ectoderm and skeleton. The endoderm of these embryos showed no further change. The ectoderm in the region of the vegetal pole tended to be more or less irregular and the shape of the body was often lumpy.

When transferred to fresh sea water after 21 hours exposure, the embryos either died or failed for the most part to continue development. The activity of the survivors increased. Blastulæ and exogastrulæ with spicules, stomodæum and apical plate were seen. Some of the survivors showed no formation of endoderm while others contained a simple endodermal tube but no skeleton. Thus the embryos were unable to overcome in sea water the poisonous effects of the metal. It acts in very low concentrations (Table I) and within a relatively short time. The effects are not reversible and embryos in a culture react differently. The various abnormal types seen in the cultures are the result of differential susceptibility among the embryos and also in the developmental processes involved. This has been a common observation in previous studies of gastrulation in the embryos of *Arbacia*.

The number of exogastrulæ decreased in concentrations from 1/10,000 to 1/6,000. Differentiation was further inhibited while

TABLE I

Effect of different concentrations of $NiCl_2$ on gastrulation and embryonic differentiation.

Control	1/60,000	1/40,000	1/20,000	1/14,285	1/10,000	1/6,666	1/5,000	1/2,857
			Oval to Spherical Blastulæ					
Gastrulæ to medium plutei	Gastrulæ to triangulars with spicules	Medium to late gastrulæ	Blastulæ to late gastrulæ. No skeleton.	More blastulæ. Movement slower. Few large simple exos.	Fewer attempts at gastrulation. Many larger simple exos, or irregular endoderm formation.	Few initial gastrulæ. Many slight exos.	No gastrulation. Few exos.	All alive. No change.
			Late Blastulæ to Initial Gastrulæ					
Medium plutei	Large triangular with skeleton. Some abnormalities. Few exos.	Somewhat smaller. Most on bottom. Few exos.	All on bottom. Blastulæ vesiculars. More exos. Few initial gastrulæ.	Blastulæ to simple gastrulæ. Few with spicules. Many exos.	Small blastulæ. Many exos. Spicules in the above.	Small blastulæ. Fewer exos. No spicules.	Blastulæ to triangular with spicules. Few exos.	All alive. No change.

growth and movement became slower. The ectoderm was the most resistant. In a concentration of 1 part in 5,000, there occurred some increase in size but no development. After 21 hours in a 1/2,000 concentration there were few intact individuals left which had not progressed beyond the state at which they were placed in the solutions.

In an effort to see how quickly the embryos would be affected, samples from a series of different concentrations were transferred at certain intervals to fresh sea water. These were examined about 24 hours later. The results are summarized in Tables II and III. They show that a relatively strong concentration of $NiCl_2$ which inhibits any further development in the experimental solution acts very quickly upon the embryo. For example, 6–7 hours exposure to a 1/2,000 concentration was fatal to further differentiation but the embryos lived and increased in size (growth) in fresh sea water.

In the tables, comparative data are given for concentrations ranging from 1/10,000 to 1/1,666. In a 1/2,000 concentration endoderm formation was entirely inhibited following an exposure of about 6 hours. In a 1/10,000 concentration the gastrulation processes first showed inhibitory effects after 7 hours in some individuals of a culture. Up to this time, the effect had been upon differentiation and growth. In several repetitions of this experiment, a peculiar result was seen. After 3–4 hours exposure followed by transfer to fresh sea water for 18–19 hours, quite normal appearing embryos were found. Longer exposure times likewise gave more active and better differentiated embryos than did the shorter exposures.

This result is surprising since it was expected that continued exposure would eventually result in death for all individuals. Is it possible that over a long exposure period the embryos lost their sensitivity to the toxic effect of the salt and were able to differentiate normally or that the salt was taken out of solution in some way? This result was observed in three out of the five repetitions. In a 1/5,000 concentration development was progressively inhibited up to about 4½ hours while after 6½ hours there was a marked advance in differentiation. In a 1/2,857 concentration this "pick up" was observed after 4½ hours exposure. In a 1/2,000 concentration the improvement was noticed in cultures exposed to the salt for 3¼ hours or longer.

In other experiments, Tables II and III, the above phenomenon was not observed. These show a progressive depression of development in the higher concentrations. In Table III it can be seen that the capacity to gastrulate was destroyed in a 1/5,000 concentration after about 8 hours exposure, and no recovery was made in fresh sea

TABLE II

Effect of exposure of blastulæ to different concentrations of $NiCl_2$ for varied periods of time. Blastulæ placed in solution and samples removed at intervals to fresh sea water. Examined 18–19 hours later. At this time the control was at a medium pluteus stage. The data show the rate and degree of toxic effect.

Solution	Hour intervals when change was made					
	1	3	4	5	6	7
1/10,000	Like control	Some retardation. Also vesicular with gut and skeleton to some with initial arm flaps.	Less retardation. Body larger and arms longer.	Stunted forms to young plutei with abnormal skeleton	All types up to young plutei; with short body and widely spaced arms.	All types up to young plutei. Gastrulæ with gut and spicules. Ectoderm lumpy in some cases.
1/3,333	Young plutei. Some with short arms or arm flaps.	Large triangular embryos with gut. Skeleton often irregular.	Large spherical blastulæ to triangular gastrulæ with spicules. Few simple exogastrulæ.	More blastulæ and fewer gastrulæ. Fewer of the latter have reached the triangular stage.	Many large, vesicular embryos. Few with flattened or irregular vegetal pole region. Few small triangulars and gastrulæ with gut and spicules.	Most are large and small vesicular embryos. More triangulars with gut, skeleton spicules. Short body.
1/2,500	Large, triangular. Skeleton often abnormal.	Same	Vesicular forms with gut	Irregular triangular with spicules. Some are vesicular with no gut.	Vesiculars. Few have gut.	Almost all on bottom. Vesiculars no gut.

TABLE II—*Continued*

Solution	Hour intervals when change was made					
	1	3	4	5	6	7
1/2,000	Large triangular embryos with gut and normal or irregular spicules. Some showed initial arm-flap formation. Ectoderm ragged in many. Body flattened.	Smaller. More vesicular. Large and small spicules. Some showed initial arm-flap formation.	Smaller and more vesicular gastrulæ. Small spciules. No initial arm flaps.	All small in size. Vesicular and irregular in outline. No skeleton. Fewer gastrulæ.	Embryos irregularly vesicular. Most show no endoderm tube, or mass of cells, or external irregularities.	Most all on bottom of dish. No internal gut. Vegetal pole region irregular in outline. A few undifferentiated and irregularly shaped exogastrula.
1/1,666	Small late gastrulæ with spicules	Small blastulæ to triangular gastrulæ with spicules. Few simple exogastrulæ.	Blastulæ to medium gastrulæ, no spicules	Mostly blastulæ. Very few showed initial gastrulation.	All blastulæ	Some showed disintegration

TABLE III

Effect of exposure of blastulæ to different concentrations of $NiCl_2$ for varied periods of time. Spherical to oval blastulæ put in solutions at a certain time and then removed to fresh sea water at intervals thereafter. These cultures examined about 24 hours later. Control showed plutei. The data show the rate and degree of the toxic effect.

Solution	Those left in in solution	Hour intervals when change was made to fresh sea water							
		2	3	4½	6	7½	9	10½	12
1/10,000	Plutei like controls	Triangular to small plutei	Same	Gastrulæ to triangular with skeleton	Small triangular with spicules	Vesiculars to gastrula	Good plutei	Same	Like control
1/5,000	Blastula to initial gastrula. Little movement	Irregular gastrula to small triangular with skeleton	Some more retardation. Spicules or none	Blastulæ to vesicular and gastrulæ. Few triangular with spicules.	Most on bottom. Irregular and lumpy in outline. More blastulæ. No skeleton.	All on bottom. More vesicular. Fewer gastrulæ.	Most are spherical blastulæ	Most are spherical blastulæ	More vesicular
1/3,333	Blastulæ to triangular gastrulæ all on bottom	Small young plutei	Vesicular to triangular with spicules. Few exos.	Same. Fewer on top	More blastulæ to large vesiculars	None on top. Blastulæ to initial gastrulæ.	Same	Same	Spherical to oval blastulæ

TABLE III—*Continued*

Solution	Those left in solution	Hour intervals when change was made to fresh sea water							
		2	3	4½	6	7½	9	10½	12
1/2,500	Spherical to oval blastulæ	Late gastrula to triangular with spicules	Many on bottom. Blastulæ to late gastrulæ with spicules.	Same. All on bottom.	Blastulæ to late gastrulæ. No spicule.	Blastulæ to slight gastrulæ	Same	Blastulæ	Same
1/2,000	Blastulæ	Blastulæ to late triangular with skeleton	Same. No skeleton.	All on bottom. Some disintegration.	Same	Blastulæ	Same	Same	Same. Some disintegration.
1/1,666	Blastulæ. Some disintegration.	Most on bottom. Blastulæ to triangular with skeleton.	Same	All on bottom. More blastulæ or slight invagination.	Blastulæ. Few initial invagination.	Same	About same	Blastulæ of various shapes	Same

TABLE IV

Effect upon development of the addition of various amounts of different metallic salts to those already present in sea water.

Metal	Control	Length of exposure	1/66,666	1/40,000	1/20,000	1/14,285	1/10,000	1/8,333	1/6,666	1/6,000	1/5,000
Al_2Cl_6 1.0%	Young plutei	13½ hrs.	Gastrula to young plutei	About same	Blastulæ to very late gastrulæ	Blastulæ to initial gastrulæ. Few top swimmers.	Fewer initial gastrulæ	Mostly blastulæ. Occasional initial gastrula.	No gastrulation. Spherical masses of cells.	Same	Some disintegration
$CdCl_2$ 1.0%	Young plutei	13½ hrs.	Gastrulæ to triangulars with spicules	Gastrulæ to early triangulars. No spicules.	Young to late gastrulæ	Blastulæ to medium gastrulæ	Occasional initial gastrulation	Young to late blastulæ	Same. Few swimmers.	Same. Some membranes persist.	Same. All in bottom. Disintegration.
				1/400,000	1/200,000	1/142,850	1/100,000	1/83,333	1/66,666	1/60,000	1/50,000
$PbCl_2$ 0.1%	Young plutei	13½ hrs.		Like control		Like control	Slight retardation	Gastrulæ to triangulars	Young to late gastrulæ	Blastulæ to medium gastrulæ	More blastulæ and fewer gastrula
$FeCl_2$ 0.1%	Young plutei	13½ hrs.	Practically no difference from the control								
			1/3,200,000		1/1,600,000	1/800,000	1/500,000	1/400,000	1/300,000	1/250,000	1/200,000
$CuCl_2$ 0.1%	Young plutei	16 hrs.	Like control		Gastrulæ to triangulars. Spicules.	Young to late gastrulæ	Initial to young gastrulæ. Some membranes persisting. Many in bottom.	Blastulæ to initial gastrulæ. Many still in membranes. Most on bottom.	Blastulæ only. Many in membranes. All on bottom.	Blastulæ only. Some disintegration.	Same. More disintegration.
$ZnCl_2$ 0.1%	Young plutei	16 hrs.	Like control		Gastrulæ to large triangulars	Late gastrulæ	Much the same. Slower movement.	Medium gastrulæ. Most on bottom.	Blastulæ to young gastrulæ	More blastulæ. Initial gastrulation.	Mostly blastulæ. Empty membranes persist.
$(ZnSO_4)$ 0.1%	Young plutei	15 hrs.	Like control		Like control	Slight retardation	Triangulars	Gastrulæ	Same	Blastulæ to young gastrulæ. Some dead.	About same
0.1% $Zn(C_2H_3O_2)_2$	Young plutei	17 hrs.	Like control		Slight retardation	Gastrulæ to young plutei	Gastrulæ	Blastulæ to young gastrulæ. Membranes persisting.	Some	Blastulæ to initial gastrulæ	About same. Large blastopore.
			1/48,000,000		1/32,000,000	1/16,000,000	1/8,000,000	1/5,000,000			
$HgCl_2$ 0.01%	Young plutei	15 hrs.	Early gastrula to triangulars without skeleton		Blastulæ to medium gastrulæ	Blastulæ to young gastrulæ	Blastulæ. Much disintegration.	Dead. Masses of spherical cells.			

water. The length of time necessary to destroy the ability to gastrulate varies according to the concentration employed; Tables II and III. The results summarized in the tables represent single experiments, but they are indicative of what was secured by several repetitions of each.

Al_2Cl_6.—In dilutions of a 1 per cent stock solution of Al_2Cl_6, gastrulation in all individuals was practically inhibited at a 1/8,333 concentration during an exposure of 13½ hours. Table IV shows that the first indication of the inhibition of gastrulation in some of the individuals was observed at a concentration of 1/20,000. Further inhibition of more individuals occurred in higher concentrations until at a 1/6,666 concentration no gastrulation took place during the experimental period. Samples transferred to fresh sea water from concentrations between 1/14,285 to 1/8,333 after this interval recovered. Gastrulation and development to the pluteus stage took place. Abnormalities of arm and skeleton formation appeared in the sample from the 1/8,333 culture. Samples transferred to sea water from the 1/6,666 culture died.

To test the rapidity of the toxic effect, samples were transferred from a 1/6,666 concentration, at certain intervals, to sea water. Those transferred after ½ to 2½ hours of exposure gave plutei. The longer exposures caused progressive retardation, and more gastrulæ and fewer plutei were found for the same length of time. After 5 hours exposure, a few gastrulated but most died in the sea water; after 6½ hours exposure, all died without any development.

When the toxic effect of a 1/5,000 concentration was tested, an exposure of ½ hour markedly retarded development, 1 hour inhibited almost all gastrulation and 2½ hours killed the embryos.

$CdCl_2$.—Table IV includes the effect of this metallic chloride in different concentrations. Over a 13½ hour period, gastrulation was inhibited in a 1/8,333 concentration. In sea water, these blastulæ grew in size, became irregular in outline, but did not gastrulate or form skeleton. Samples from a 1/10,000 concentration formed large, vesicular, irregular blastulæ and gastrulæ in sea water.

$PbCl_2$.—Lead chloride is slightly more toxic than the three metallic chlorides just mentioned, and hence a 0.1 per cent stock solution was used. Table IV includes part of the data secured in one experiment which may be considered typical. Slight retardation became evident in a concentration of 1 part in 100,000. In a concentration of 1/60,000 gastrulation was inhibited in some individuals. The latter increased in number in a concentration of 1/50,000 after which little change was noted in the cultures until a concentration of 1/35,714 was reached.

One result was the persistence of the fertilization membranes even after the embryos had escaped. Thus this salt causes the membranes to harden and prevents their dissolution during the experimental period.

In the 1/35,714 solution late gastrula to triangular stages were found, while in a 1/33,333 concentration even young plutei occurred. This condition was found in all cultures up to a concentration of 1/22,222. No higher concentrations were tried. This peculiar change may perhaps be accounted for by the precipitation of lead seen in the bottom of the container, during the experimental period. The precipitation did not appear when the experimental solutions were made up, but only after standing. Enough was left in solution to retard development slightly.

$FeCl_2$.—This metallic chloride is apparently not toxic to any extent. Very little if any difference was observed in graded cultures up to a concentration of 1 part in 14,285.

$CuCl_2$.—This metallic salt is far more toxic for development than $PbCl_2$. A 0.1 per cent stock solution was used. Table IV summarizes the results of a typical experiment. Gastrulation was inhibited in most individuals in a concentration of one part in 400,000 and entirely in a 1/300,000 concentration. The fertilization membranes showed a hardening in these concentrations and failed to dissolve. This must occur rather quickly because in these and higher concentrations more of the embryos failed to escape from the membranes. The same persistence of the membranes was also seen in the $PbCl_2$ solutions but to a lesser extent. In the latter case the embryos had escaped so the hardening occurred more slowly. This lack of digestion of the membranes in both the $PbCl_2$ and $CuCl_2$ solutions may be accounted for by enzyme-poisoning which is characteristic of some of the heavy metal salts. As will be seen below, the higher concentrations of the Zn salts likewise produced persistent membranes.

After transfer to fresh sea water, the original 1/500,000 culture continued development to the pluteus stage. Also large vesicular blastulæ without skeleton were found, indicating growth of the ectoderm but inhibition of endoderm and mesoderm formation. Most of the individuals in the 1/400,000 concentration gastrulated in fresh sea water and in 24 hours formed triangular embryos with spicules. More large blastulæ were present as well as a few exogastrulæ showing tri-partite gut, anal opening, and apical plate. Thus in some individuals this metallic salt inhibits the gastrulation processes without inhibiting endoderm formation.

As seen in Table IV, no gastrulation occurred in a 1/300,000 concentration during the experimental period. However, in sea water some did gastrulate and a very few formed simple exogastrulæ. No skeleton was seen in any individual, indicating the inhibition of mesoderm formation or the formation of skeleton during the period of observation. The endoderm did not differentiate. Only a few individuals from a 1/250,000 concentration recovered in sea water. Large, spherical and oval blastulæ were found as well as a few initial gastrulæ. The individuals from a 1/200,000 culture failed to gastrulate even after 48 hours; many died; and many failed to get out of the membranes.

The time required for recovery varies of course with the concentration. Embryos which did not gastrulate or in which no skeleton formed during a 24-hour period in sea water, did so after 48 hours. Since it is known that certain marine organisms may store copper in their tissues (oysters) this inhibition may be due to the concentration of the toxic metal, and the slowness of recovery and differentiation be due to the slowness with which the metal left the cells and again reëntered the sea water in such a dilute concentration as not to inhibit further development. The metal could not have irreversibly injured the protoplasm, but rather inhibited its function.

Angerer (1935) has found that after *Arbacia* eggs were exposed to $CuCl_2$ an interval of time elapsed during which the metal produced no visible effect in the protoplasm as regards its viscosity. This time interval is a function of the concentration of copper in solution. At the conclusion of this latent period, there ensues a sharp rise in viscosity values resulting in an irreversible gelation (coagulation) of the protoplasm. In the case of *Arbacia* egg protoplasm, there is no concentration at which gelation is reversible.

In the light of Angerer's results the temporary inhibition of differentiation cannot have been due to gelation, otherwise recovery would not have occurred. An irreversible effect was seen first in the concentration of 1/200,000 where no recovery occurred even during 48 hours in sea water.

To test the rate of lethal action of $CuCl_2$, a concentration of 1 part of stock solution in 150,000 parts sea water was used, which inhibits all development and causes complete disintegration in an 18-hour period of exposure. Embryos were removed from this solution to fresh sea water at various intervals. An exposure of 3½ hours resulted in some inhibition and many persistent membranes from which numerous embryos had failed to escape. After 9½ hours in the solution only a few embryos gastrulated during the following 28 hours in sea water. Those which did not escape from the membranes, or were only partially successful in it, did not differentiate. A concentration of 1/250,000 produced no inhibitory effects during 8 hours exposure. Membranes hardened while the embryos which did not escape differentiated slightly.

$HgCl_2$.—This metallic chloride is very toxic. A concentration of 1 part in 48,000,000 parts of sea water retarded development while a concentration of 1/8,000,000 prevented gastrulation during a 15-hour exposure (Table IV). After return to sea water, only very few of the more hardy individuals were able to gastrulate, but they developed no further. In a concentration of 1/5,000,000, the swollen cells showed a clumping of the pigment granules.

To test the rate of action of $HgCl_2$, spherical to oval blastulæ were placed in a concentration of 1/2,500,000. The embryos transferred to sea water after 5 minutes exposure were able to recover with only slight evidences of retardation in 24 hours. A 10-minute exposure visibly retarded development. More gastrula and triangular stages were found, while the control showed medium plutei. After 15-minute exposure, fewer individuals were able to gastrulate. These decreased in numbers following exposures of 20 to 45 minutes. During this interval more of the embryos were killed and movement became progressively slower. An exposure of 55 minutes killed most of the embryos, though a few survivors showed attempts to gastrulate.

This metallic salt does not appear to affect the gastrulation process independent of other developmental processes. As long as any survived they still attempted to gastrulate. Development went no further, however, during the period of observation. The most pronounced toxic effect on the majority of the embryos occurred within the first 15 minutes exposure.

$ZnCl_2$.—This metallic salt is slightly less toxic than $CuCl_2$. Gastrulation was inhibited in most individuals when the concentration was about 1/200,000. The most pronounced inhibitory effects appeared in concentrations up to 1/400,000. Persistent membranes appeared in the higher concentrations, and embryos failed to escape. Also an increasing number of embryos were killed in concentrations of 1/120,000 and higher. In these concentrations only an occasional attempt at gastrulation was seen; while in a 1/100,000 concentration, no attempt at gastrulation was seen and many were dead. On return to fresh sea water, the surviving individuals formed large globular structures without gut or skeleton. The ectoderm was often irregular and lumpy in appearance and movement was lethargic.

To test the rate of action of $ZnCl_2$ samples were transferred to fresh sea water from a 1/10,000 dilution every five minutes following their introduction at 6:50 P.M. and were examined the following morning at 8–9 A.M. The control at this time showed young plutei. A 5-minute exposure gave blastulæ to late gastrulæ. Membranes persisted and some of the embryos failed to escape completely. The latter had not gastrulated. A 15-minute exposure gave blastulæ to young gastrulæ. Many had died and disintegrated or failed to escape from their membranes. The toxic effect of this metallic chloride is manifested very quickly after exposure. The most pronounced effects occurred within the first 10–15 minutes. After this the noticeable changes occurred very gradually. Thus even after one-half hour of exposure, gastrulation of many of the surviving embryos did occur during the experimental period. Movement, however, was very lethargic. After 1-hour exposure no normal gastrulæ were found, although some had attempted it.

$ZnSO_4$.—A stock solution of 0.1 per cent was used. As seen in Table IV, a concentration of 1/500,000 caused conspicuous retardation. In a 1/250,000 concentration, some individuals failed to gastrulate and those which did gastrulate were still in the young gastrula stage after 15 hours exposure. In a concentration of 1/175,000 most failed to gastrulate and some died. The hardened membranes failed to dissolve. The few individuals which attempted to gastrulate showed very broad invaginating regions. This effect was also observed in the $ZnCl_2$ study. In a 1/100,000 concentration only an occasional individual showed an attempt to gastrulate.

Zn Acetate.—This salt gave results quite comparable to the other zinc salts used (see Table IV). It caused the membranes to persist and the initial gastrulæ in the higher concentrations showed broad blastopores. The blastocœl was packed with cells. Gastrulation seemed to be inhibited at concentrations of 1/100,000 and higher.

V

It is well known that cyclic changes in the distribution of marine invertebrates are often accompanied by changes in the chemical composition of the sea water. Reproduction, embryonic development and growth are dependent on the presence of various constituents but the relative proportions of the various necessary elements may vary without any essential detrimental results. The absence of a necessary element or its presence in a non-utilizable form naturally disturbs development while the presence of too much of the element (experimental study) will also bring about developmental changes. The latter probably does not play as important a part in marine life as does the lack of the necessary amount of the element, but nevertheless the study furnishes information on the specific effect of the element upon development.

The chlorides of some heavy metals, especially $NiCl_2$, whose effects on gastrulation and subsequent differentiation in the sea urchin, *Arbacia punctulata*, have been described in this study, may be added to the growing list of physical and chemical agents which may provoke exogastrulation. As is well known from studies by others, some metals are more toxic than others (Table IV) and the more toxic ones exert their toxic effect on gastrulation very quickly. The relative toxicity of the metallic salts seems to be as follows: $HgCl_2 > CuCl_2 > ZnCl_2$, $ZnSO_4$, $Zn(C_2H_3O_2)_2 > PbCl_2 > Al_2Cl_6$, $CdCl_2$, $NiCl_2 > FeCl_2$. In the case of most of the salts employed, the initial effect is upon growth and differentiation. Retardation and inhibition become increasingly more conspicuous in progressively higher concentrations, while the gastrulation process is the last to be effected. Finally it has been found that certain of the metals, especially $NiCl_2$, give a larger number of exogastrulæ at certain concentrations than in some of the previous studies where other types of environmental modifications were made, but they are not as effective in provoking exogastrulation as $LiCl_2$ (Runnström). It would appear, therefore, that such different physiological processes as gastrulation, differentiation and growth have different thresholds of inhibition for the same toxic substance.

Information is given on the concentrations which inhibited gastrulation. Providing the exposure has not been long enough to injure the embryo fatally, gastrulation and even further development will take place in fresh sea water. The rate at which some recovery takes place depends upon the concentration employed and the length of exposure. No cases of fused embryos were found.

As seen in previous studies (*cf.* Runnström, Lindahl), differentiation of the ectoderm and even of mesoderm, within limits, may take place independently of gastrulation or of the formation of the gut tube. Of the three germ layers, ectoderm is the most resistant to injury. In general the types of inhibitory or retardational effects are similar to those produced by other environmental modifications and by other workers. These include the behavior of the skeleton forming mesenchyme, the development of body form and size, the relative proportions of the potential ectoderm and endoderm, the inhibition of the gastrulation process, etc. The various metals give quite similar results but at different concentrations.

Information is also given on the rate of action of a lethal solution of each metallic chloride tested. The more toxic the metal, the quicker are inhibitory effects shown even in very dilute concentrations. In higher concentrations of certain heavy metals like Zn, Cu and Pb, the fertilization membrane does not disappear. In such cases, and even where partial escape has occurred, the embryos do not differentiate. This lack of digestion of the membrane may be due to enzyme-poisoning which is so characteristic of some of the heavy metal salts (Ishida, 1936).

Although the differentiation of organs and tissues in both gastrulated embryos and in those where exogastrulation has occurred has been of interest to numerous workers, the effect of environmental modifications on the relative proportions in the amount of ectoderm and endoderm has attracted much study (Runnström, Lindahl, Lindahl and Stordal, and others). By appropriate stimulation of the egg the embryo can be animalized or vegetalized and the differentiation followed. Furthermore, this phenomenon can be inhibited by various means. For example, Li salts will vegetalize the embryo while SO_4 deficiency will animalize (ectodermize) the embryo.

Chlorides of certain heavy metals, acting on the blastula stage, likewise produce ectodermal embryos which lack gut or any endodermal material as far as can be seen. All transitions between this and typical gastrulation occur. The ectodermal embryos differentiate skeletal spicules or rods, apical plate and sometimes stomodæum. If treatment has not been too severe, the exogastrulæ likewise differentiate these structures. Arms never develop but oral and anal flaps may appear and the body tends towards typical shape which, however, is modified by the abnormal distribution of skeletal material. In extreme cases only large globular, ectodermal embryos are found in which the skeleton does not go beyond the spicule stage and neither stomodæum or apical plate differentiates.

Interpreted in the light of Runnström's hypothesis, the heavy metals employed may effect in a differential manner the ectodermal and endodermal gradients which he believes to exist in the fertilized egg (ectodermization and endodermization of the embryo, Lindahl, 1936). It has been shown previously that these same gradients may be present at the blastula stage and hence the limits of the endoderm and ectoderm are not definitely established even at the oval blastula stage which immediately precedes gastrulation. It is doubtful if the limits are established even at gastrulation. Lindahl (1936) considers that the two gradients have different metabolic rates and also that hydrocarbon metabolism dominates the animal pole while protein metabolism dominates the vegetal pole. The heavy metals used in this study may therefore exert their toxic action upon these metabolic processes or produce substances which give the same effect which would account for some of the various developmental abnormalities and inhibitory effects described above.

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THE CYCLE OF ORGANIC PHOSPHORUS IN THE GULF OF MAINE

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It is generally recognized that the fertility of the sea depends upon a cycle in which carbon, nitrogen, phosphorus and other substances are assimilated under the influence of photosynthetic processes in surface waters and are set free again by processes of digestion or decay. The total organic productivity of a region is limited to the rate at which this cycle is completed. While it is apparent that in many localities the principal limiting factor is the rate of restoration of the inorganic products of decay (NO_3, PO_4) to the surface or photosynthetic zone, little is known concerning the exact locus within the sea at which decomposition actually sets these substances free, or of the rate at which the cycle as a whole or in part is completed.

The concentration of the ultimate products of decomposition, such as NO_3 and PO_4, tell us little about these points since they are stable substances capable of accumulating over a long period of time to high concentrations, and of being transported far from their place of origin. The actual site of decomposition is better indicated by the presence of intermediate products of decay such as ammonia, nitrite, and organic compounds of nitrogen and phosphorus.

The present paper is an account of the distribution of phosphorus throughout the year at a standard station in the Gulf of Maine. At all depths the amount of phosphorus present has been measured in three forms: (1) inorganic phosphate (PO_4), (2) dissolved organic phosphorus, and (3) particulate organic phosphorus. The analysis of these data gives some indication of the magnitude of the synthetic and disintegrative processes undergone by phosphorus compounds in different parts of the water column at different times of year, and of the extent to which phosphorus is transported from one depth to another.

The station chosen for study was located in the deeper portion of the western basin of the Gulf of Maine, 30 miles northeast of Highland Light. The surface water in this region is apparently relatively free from strong non-tidal currents, being sufficiently far offshore to avoid the coastal drift which accompanies the freshening of the water along the margin of the Gulf. In this part of the Gulf, as the result of freedom from strong currents, there develops each summer maximal surface temperatures and maximal stability of the water column. In this location one also finds relatively deep water (230–270 meters) free from strong non-tidal currents. Preliminary measurements made by Dr. E. E. Watson with current-meter indicate maximal tidal velocities of 11 cm. per second at 40 cm. above the bottom, and 14 cm. per second at 177 cm. above the bottom. The oxygen content in the deep water below 200 meters is low, varying from 4 to 4.5 cc. per liter. The point chosen for study thus presents conditions in which there exists above the bottom a considerable column of water too poorly illuminated to permit of photosynthesis, which terminates at a depth of 40 to 50 meters (Clarke and Oster, 1934) and in which decomposition occurs in sufficient magnitude to maintain a low oxygen concentration, and one as well chosen as may be to avoid disturbances due to the non-tidal drift of the water.

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[1] Contribution No. 127.

TABLE I

Station positions and dates.

Atlantis Station Number	Date	Location	Depth of water
			meters
2440	May 18, 1935	42° 22′ N. 69° 35′ W	249
2468	August 20, 1935	42° 20′ N. 69° 32′ W	232
2493	November 8–9, 1935	42° 21.5′ N. 69° 32′ W	256
2495	February 26, 1936	42° 22′ N. 69° 33′ W	270
2558	May 14, 1936	42° 27′ N. 69° 31.5′ W	254

Stations were made on five occasions separated by intervals of three months, thus completing one yearly cycle. Their positions and dates are given in Table I. The approximate position is indicated by a circle in Fig. 1.

ANALYTICAL PROCEDURE

Inorganic Phosphate

Phosphate samples were collected in black bottles and analyzed for inorganic phosphate at room temperature on shipboard by the Deniges-Atkins method, except that the solution of stannous chloride used contained 0.1 gram $SnCl_2.2H_2O$ in 10 ml. of 1 to 10 hydrochloric acid. Salt effect correction factor as measured was 1.35. Corrections for salt error and reagent blank were both applied.

Particulate Organic Phosphorus

Samples of water of about 300 ml. volume were filtered on shipboard as soon after collection as possible.

The procedure by which particulate organic phosphorus was determined is as follows. The particulate matter is filtered out by suction on a precipitate of barium sulfate on a 3G4 Jena sintered glass funnel with polished surface. The barium sulfate precipitate is prepared by stirring 0.6 ml. of normal barium chloride into about 10 ml. of hot water containing excess sulfuric acid. It is poured over the funnel, the liquid sucked through, and filter and flask washed thoroughly with distilled water. The sea water sample is run through the

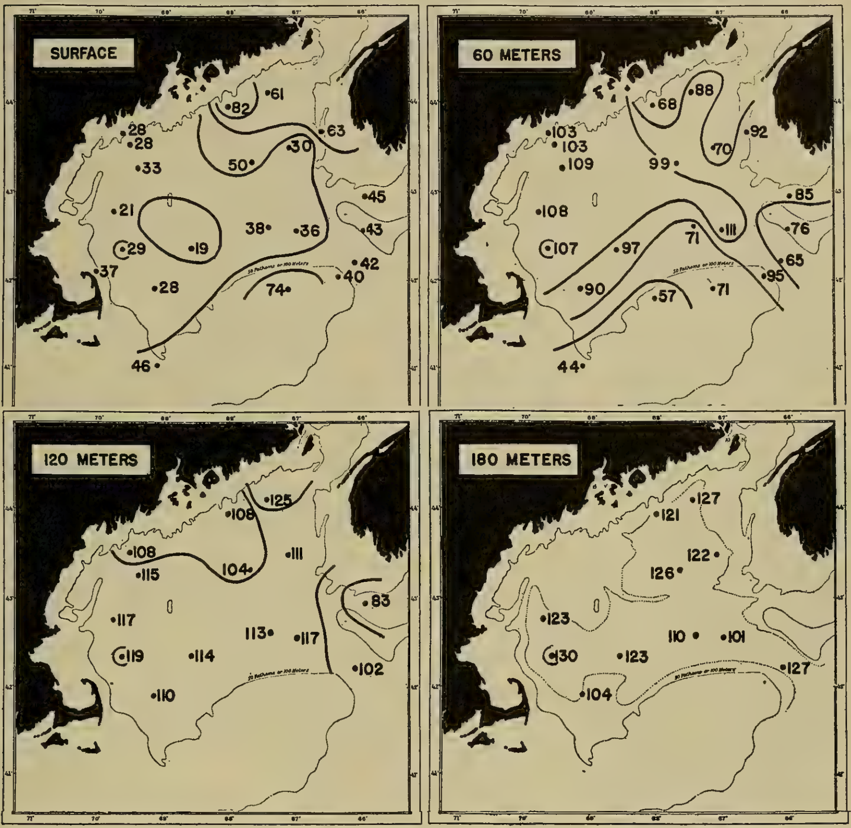
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FIG. 1. The distribution of inorganic phosphate, PO_4, throughout the Gulf of Maine in May, 1934, at the surface and at the depths of 60, 120, 180 meters. The circle indicates the position of the stations at which the present investigations were made.

filter, and the volume of the filtrate measured. Plankton and barium sulfate are washed off the filter into a 125-ml. Erlenmeyer flask with a stream of distilled water. The sides and bottom of the filter are cleaned with a rubber policeman and all the precipitate transferred to the flask to insure removal of the plankton. The funnel is cleaned by reverse suction, treatment with sulfuric and chromic acids and thorough washing.

After the addition of 2 ml. of 38 per cent (by volume) sulfuric acid to the plankton, the flask is evaporated on the steam-bath to charring of the organic matter. The flask is ignited to fumes of sulfuric anhydride, and a drop of phosphate-free hydrogen peroxide (Cooper, 1934) (prepared by vacuum distillation of concentrated hydrogen peroxide) is added. A few seconds heating without loss of sulfuric anhydride fumes suffices to destroy the organic matter. The excess hydrogen peroxide is decomposed by heating the sample at 120° C. for one hour on an oil-bath. The cooled samples are diluted with ca. 50 ml. of distilled water and warmed on a steam-bath before filtering through a sintered glass funnel to remove the barium sulfate. The filtrate is diluted to 100 ml. in a glass-stoppered bottle. After the addition of 2 ml. of $2\frac{1}{2}$ per cent ammonium molybdate each sample is shaken. Standards of similar phosphate content as potassium dihydrogen phosphate are made up with 2 ml. of 38 per cent sulfuric acid, 2 ml. ammonium molybdate and distilled water to 102 ml. and shaken. Two drops of stannous chloride solution containing 2.5 grams $SnCl_2.2H_2O$ in 100 ml. of 1 to 10 hydrochloric acid are added to each sample and standard with immediate shaking. After five minutes the samples are compared with the standards in a colorimeter with about 30 cm. depth of solution. Phosphate in the reagents for the development of the color (designated as Blank A) is determined by intercomparison of dilute standards. Phosphate introduced in the treatment of the unknowns (designated as Blank B) is determined by carrying through the procedure on a barium sulfate precipitate not treated with sea water. Blank A is added to the standards to give their true phosphate content. Both Blanks A and B are subtracted from the phosphate found in the unknowns. The net phosphorus in grams $\times 10^{-6}$ found in the unknowns multiplied by $\frac{1{,}000}{\text{ml. sample}}$ gives the plankton phosphorus in grams $\times 10^{-6}$ per liter. Errors in the analysis due to loss of phosphorus by volatilization or change in acidity by loss of sulfuric anhydride are shown to be negligible by carrying through blank analyses with known amounts of added phosphorus.

A procedure employing nitric acid as the oxidizing agent (Robinson and Kemmerer, 1930), in which the nitric acid was evaporated off and the last traces removed by an evaporation with hydrochloric acid gave check results with the hydrogen peroxide procedure but a poor color match with the distilled water standards. It might be possible to use a procedure similar to that of von Brand (1935) for separation of the diatoms from sea water. No effort was made to differentiate between phosphorus and arsenic in the plankton phosphorus determinations. It was found, however, that the procedure of Zinzidze (1935) using bisulfate and sulfuric acid will bring about the complete reduction of at least 9×10^{-6} grams of arsenic to the limit detectable by the cerulio-molybdate method (2×10^{-7} grams) and make possible a distinction between phosphate and arsenic in plankton analyses.

Dissolved Organic Phosphorus

The phosphorus in solution in organic combination was obtained by subtracting the inorganic phosphorus from the total phosphorus in the filtrate from which the particulate matter had been separated. The following procedure for the determination of total phosphorus in a sea water sample was devised with a view to avoiding the interference of pentavalent arsenic with the ceruleo-molybdate phosphorus determination and to insure the complete destruction of organic matter. In the more rapid methods of analysis which have been used, in which sea water is treated directly with oxidizing agents, arsenic in the sample is oxidized and not subsequently reduced, while organic matter is not completely destroyed in solutions containing large amounts of chloride.

Fifty-milliliter samples of sea water are treated with 3 ml. of concentrated sulfuric acid (arsenic-free) in a 125-ml. Erlenmeyer flask. After evaporation on a steam-bath under a hood to carbonization of the organic matter, each sample is evaporated to the formation of fumes of sulfuric anhydride with swirling to avoid bumping. A drop of phosphorus-free 5 per cent hydrogen peroxide (prepared by vacuum distillation of 30 per cent hydrogen peroxide) is added. The flask is heated in the same manner without loss of sulfuric anhydride for half a minute more to make the solution colorless. If necessary, more hydrogen peroxide may be added. About 60 ml. of distilled water is added to the cooled sample, which is set on the steam-bath for complete solution of the precipitated salts. After transfer to a 500-ml. Erlenmeyer and addition of 8.5 ml. of concentrated ammonia water, the excess of ammonia is boiled off and the volume of solution reduced to about 30 ml. The sample is transferred to a small ground-glass-stoppered bottle and warmed on the steam-bath, open, with 2.47 ml. of 38 per cent (by volume) sulfuric acid. Four milliliters of 10 per cent sodium sulfite (anhydrous, or hydrated salt in double the concentration) is added, and the stopper held in with a clip to prevent the escape of sulfur dioxide. Excess hydrogen peroxide is immediately destroyed, but eight hours heating on the steam-bath is necessary to reduce pentavalent arsenic to the trivalent form. The sample is transferred to a 500-ml. Erlenmeyer, boiled for five minutes to remove sulfur dioxide, cooled, and diluted to 100 ml. in a ground-glass-stoppered bottle. The residual acid in the sample is 2 ml. of 38 per cent sulfuric. After the addition of 2 ml. of 2.5 per cent ammonium molybdate the sample is shaken. Standards which will develop about the same color intensity as the unknowns are made up from a potassium dihydrogen phosphate solution with the addition of 2 ml. 38 per cent sulfuric acid, 2 ml. of 2.5 per cent ammonium molybdate solution, and distilled water to 102 ml. These are shaken. Each standard and sample is again immediately shaken after the addition of two drops of a solution containing 2.5 grams of $SnCl_2$ $0.2H_2O$ in 100 ml. of 1 to 10 hydrochloric acid. After four or five minutes for the development of the color the samples are compared with the standards in a colorimeter with about 30 cm. depth of solution.

Intercomparison of dilute standards in distilled water is made to estimate the phosphate in the reagents for producing the color (designated Blank A). A blank determination carried through with reagents alone gives the phosphate introduced by reagents in the treatment of the sample (designated Blank B). Blank A is added to the standards to give their true strength in calculating the phosphate in the unknowns. Both Blanks A and B are subtracted from the phosphate found in the unknown. The salt effect correction has been determined as 0.885 for unknowns by analysis of the same sea water with varying amounts of phosphate added. The slope of the graph of phosphate added against phosphate recovered is the salt effect correction. No variation in salt effect correction was found with salinity from 31.5 to 38.5. A similar procedure gave a salt effect correction of 0.81 in determining Blank B. Change of salt effect correction with temperature was not studied but extremes of temperature were avoided in making the analyses.

Since several samples of sea water gave identical analyses with or without the addition of as much as 165 mg. per cubic meter of arsenic (about eight times the amount found in sea water), arsenic was assumed to be reduced completely by the procedure used. Reduction of arsenic in solutions for determining Blank B was found to be much slower than in sea water samples and quantitative only for amounts of added arsenic equivalent to 50 mg. per cubic meter or less. The difficulty was avoided by the use of reagents containing negligible amounts of arsenic. Schering-Kahlbaum "pro analysi" sulfuric acid was found suitable. The method can easily be adapted to use with a photometer although this was not attempted.

Table II, containing representative data from the analysis of samples of sea water, illustrates the procedure used in computing the dissolved organic phosphate, and gives some indication of the dependability of the methods. The total phosphorus in the samples obtained by combining the phosphorus in the filtrate with the particulate phosphorus retained by the filter (column IV) is compared with the total phosphorus in the water obtained by direct analysis without filtration (column V). It is evident that some small losses result from filtration, but these do not greatly exceed the normal variation of the Deniges-Atkins method. The values for the particulate phosphorus are small, but are consistent within themselves. The smaller values do not greatly exceed that of the blanks employed and in one set of data (for Station 2468, August 20, 1935) the values for the particulate phosphorus are in doubt owing to uncertainty in the value of the blank. The values for dissolved organic phosphorus in these samples are consistently positive and larger than the apparent errors of the method. During a large part of the year, as will be shown, much less soluble organic phosphorus is present in the water. Since this fraction is obtained by taking the difference of two large measures, each subject to considerable errors, it sometimes eventuated at such times that negative values were obtained for this fraction. The methods employed evidently do not give an exact measure of the distribution of the phosphorus fractions, but they do serve to indicate the general magnitudes of the quantities in which each occurs.

The meaning of the particulate and dissolved fractions of organic phosphorus depends upon the properties of the barium sulfate filter. When a suspension of diatoms, *Nitzschia closterium*, containing a known amount of phosphorus is filtered, the phosphorus may be recovered quantitatively from the filter. The refiltration of a filtrate leaves no detectable amount of phosphorus upon the filter. Evidently diatoms and microorganisms of similar size are completely retained in the particulate fraction. Dr. Charles E. Renn has kindly tested the filter for the retention of bacteria. After filtering sea water containing some 400 bacteria per milliliter the filtrate contained about one-sixth that amount. The particulate organic phosphorus fraction probably contains the phosphorus of all the larger phytoplankton and smaller zoöplankton and the greater portion of the bacteria and detritus as well. The dissolved organic phosphorus fraction may include a small

TABLE II

Specimen of data on phosphorus fractions in samples of sea water. Concentrations expressed as milligrams PO_4 per cubic meter.

Depth in meters	I Inorganic phosphorus	II Particulate phosphorus	III Phosphorus in filtrate	IV Total phosphorus II + III	V Total phosphorus. Direct analysis	VI Difference	VII Organic phosphorus III − I
1	45	10	64	74	62		
			70	80	86		
			68	78			
			67	77	71	+ 6	22
10	27	13	64	77	68		
			60	73	86		
			64	77			
			63	76	77	− 1	36
20	39	14	66	80	86		
			70	84	86		
			45	59			
			60	74	86	−12	21
60	110	6	116		130		
					116	− 1	6
				122	123		
100	110	4	126		142		
					142		
				130	142	−12	16
125	115	5	132		138		
					146		
				137	142	− 5	17
					160		
					156		
150	111	5	142	147	158	−11	31
200	123	5	152		154		
					162		
				157	158	− 1	29
225	108	8	148		162		
					158		
				156	160	− 4	40

portion of the bacterial flora, perhaps some minute nannoplankton, and detritus which has been reduced to the smallest dimensions in addition to organic compounds of phosphorus in colloidal form or in true solution. Renn (1937) has estimated that a bacterial population of 100,000 cells per milliliter would represent only 2.9 mg. PO_4 per cubic meter. It is evident that the much larger values of dissolved organic phosphorus obtained in our analyses can be due in only negligible part to the presence of bacteria. We believe, consequently, that our measurements represent chiefly the presence of phosphorus compounds in solution.

TABLE III

Inorganic phosphorus. Concentrations expressed in milligrams PO_4 per cubic meter. Depths not corrected for wire angle, which in no case would reduce by more than 4 per cent.

Depth in meters	May 18 1935	August 21 1935	November 8–9 1935	February 26 1936	May 14 1936
1	35	5	45	105	14
10	28	21	27	102	10
20	34	53	39	103	15
30	59	96	78	99	82
40	70	92	73	100	95
50	—	—	77	96	97
60	97	93	110	88	116
80	105	110	89	99	117
100	108	109	110	95	120
125	114	107	115	99	129
150	122	122	111	96	147
175	138	132	123	131	152
200	146	137	123	141	146
225	161	136	106	143	157
250	170	139		139	

The distribution of phosphorus in the three forms into which it has been separated is recorded in Tables III, IV, V and VI. Inorganic phosphate represents by far the greater quantity of phosphorus in the water amounting to 72 to 92 per cent of the total at different times. It is rather uniformly distributed at depths greater than 80–100 meters throughout the year, the concentrations increasing somewhat with depth. In the superficial layers, as has been frequently observed elsewhere, the inorganic phosphate becomes greatly reduced in quantity in the spring and is restored to concentrations characteristic of greater depths during the winter.

Particulate organic phosphorus, representing organisms and detritus, is in general the smallest of the three fractions, amounting to about 5 per cent of the total. It occurs in greatest quantity in the upper layers—above 40 meters—corresponding to the observed distribution of phytoplankton (Gran and Braarud, 1935). The quantities in these layers are highest in spring; in midwinter the quantity in surface water is scarcely to be distinguished from that in deep water. The values obtained in August are subject to doubt. Below the photosynthetic zone the distribution of filterable organic phosphorus is on the whole very uniform, amounting to about 5 γ PO_4 per liter. The distribution and magnitude of the concentrations of filterable phosphorus agree well with that of particulate nitrogen observed by von Brand (1937) in these waters in the summer of 1936.

TABLE IV

Particulate organic phosphorus. Concentrations expressed in milligrams PO_4 per cubic meter. Depths not corrected for wire angle, which in no case would reduce by more than 4 per cent.

Depth in meters	May 18 1935	August 21 1935	November 8–9 1935	February 26 1936	May 14 1936
1	20	13	10	6	17
10	18	14	13	5	13
20	15	15	14	5	20
30	20	7	9	4	12
40	12	8	7	4	0
50	—	—	5	4	8
60	5	5	6	6	9
80	8	6	4	4	12
100	5	5	4	4	3
125	4	3	5	3	2
150	3	—	5	3	3
175	5	3	13	3	1
200	4	3	5	3	7
225	4	4	8	4	7
250	6			5	

In May dissolved organic phosphorus occurred in only minimal quantities in the water of all depths except near the surface. During the summer the concentration increases markedly until November, when over 20 per cent of the total phosphorus is in this form. In the early winter there is a rapid disappearance of this form of phosphorus, associated in time with the increase in concentration of inorganic phosphate in the upper waters. The appearance of organic phosphorus commences in May at the surface, and the concentrations appear to grow from the surface downward. Not until November are high concentrations observed near the bottom. These observations suggest that considerable decomposition is taking place throughout the water column and in particular in those depths where phyto- and zoöplankton are known to exist in greatest numbers; and that at this station decomposition at the bottom may be relatively unimportant. The findings concerning organic phosphorus are somewhat similar to those of Kreps and Osadchik (1933), who made studies in Barents Sea. They found organic phosphorus to show a gradual increase from August to January reaching concentrations of some 40 mg. P_2O_5 per cubic meter and actually exceeding the inorganic phosphorus during the latter month. Their observations, which did not cover the earlier part of the year, showed the greatest concentrations in the deeper waters. As will be pointed out in a subsequent publication, the seasonal distribution of soluble organic phosphorus has some resemblance to that of ammonia, which may be considered to be a somewhat analogous stage in the nitrogen cycle.

TABLE V

Dissolved organic phosphorus. Concentrations expressed in milligrams PO_4 per cubic meter. Depths not corrected for wire angle, which in no case would reduce by more than 4 per cent.

Depth in meters	May 18 1935	August 21 1935	November 8–9 1935	February 26 1936	May 14 1936
1	30	49	21	−2	12
10	15	36	35	0	0
20	−1	50	29	22	35
30	−4	14	7	14	18
40	3	24	36	−3	3
50	—	—	43	5	3
60	8	36	7	1	26
80	0	24	50	9	55
100	2	30	24	21	22
125	−3	29	20	21	22
150	2	6	38	22	9
175	−6	20	20	4	−11
200	−7	22	30	6	8
225	−2	17	43	−4	10
250	8			−10	

The data recorded in Tables III, IV and V have been submitted to further analysis with a view to determining in so far as possible, just what alterations take place in the phosphorus cycle at various depths and at different times of year.

The column of water is considered to be virtually a closed system in which every exchange with the surroundings is exactly balanced by an equal and opposite exchange. By dividing the column into a number of segments lying at different depths, in which the quantity of phosphorus in the different forms is recorded from time to time, and by observing certain general biological and hydrographic principles, it becomes possible to estimate to what extent changes in the concentrations of each form of phosphorus may be derived from processes taking place in situ, and to what extent vertical movements of phosphorus from one segment or layer to another must be postulated.

To obtain a workable body of data, Table VI has been drawn up recording the quantity of phosphorus present in each of the three forms for each of four layers each of 60 meters depth at each time of observation. The values are obtained by graphical integration and are expressed as percentages of the total phosphorus in a water column of 240 meters depth at each time of observation. In analyzing these data the following premises are held:

TABLE VI

Summary of distribution of phosphorus fractions.

	Depth in meters	May 18 1935	Aug. 20 1935	Nov. 8 1935	Feb. 26 1936	May 14 1936
Total P as grams PO_4 per sq. m.	0–240	28.7	31.0	32.9	29.9	34.4
Percentage of total phosphorus in each 60-meter layer	0–60	16.4	20.6	18.0	22.0	14.9
	60–120	23.7	23.2	25.0	23.0	28.0
	120–180	27.0	26.3	28.0	25.0	27.9
	180–240	32.9	29.9	29.0	30.0	29.2
	Total	100.0	100.0	100.0	100.0	100.0
Inorganic phosphorus as percentage of total	0–60	11.9	12.6	11.3	19.7	10.6
	60–120	22.1	16.8	18.7	19.4	20.8
	120–180	26.0	22.5	21.6	21.1	25.0
	180–240	32.0	25.6	21.2	28.7	26.5
	Total	92.0	77.5	72.8	88.9	82.9
Dissolved organic phosphorus as percentage of total	0–60	1.5	6.2	5.0	1.4	2.3
	60–120	0.4	5.4	5.4	2.7	6.0
	120–180	0.2	3.2	5.0	3.3	2.5
	180–240	0.0	3.7	6.4	0.6	1.7
	Total	2.1	18.5	21.8	8.0	12.5
Particulate organic phosphorus (organisms and detritus) as percentage of total	0–60	3.0	1.8	1.7	0.9	2.0
	60–120	1.2	1.0	0.9	0.9	1.2
	120–180	0.8	0.6	1.4	0.6	0.4
	180–240	0.9	0.6	1.4	0.7	1.0
	Total	5.9	4.0	5.4	3.1	4.6

1. *The horizontal exchange due to the drift of water past the station may be neglected.* This premise is not justified on the ground that the

station is located in a region of minimal drift. There can be little doubt that water is constantly drifting past the station. The observed changes in salinity demonstrate this. The total phosphorus recorded varies ± 7 per cent from the mean value throughout the year. Since this variation shows no seasonal sequence, the total phosphorus being lowest in May, 1935 and maximal in May, 1936, there evidently is some variation in the character of the water occupying the station at different times. These differences are eliminated by expressing the phosphorus fractions as percentages of the total, a procedure which imposes artificially the character of a closed system upon the set of data. The justification of this procedure lies in the relatively small differences in total phosphorus observed from time to time, and in the fact that on the whole the horizontal distribution of phosphorus throughout the Gulf at any time, at least to judge by inorganic PO_4, is much more uniform than is the vertical distribution. The horizontal distribution of PO_4 at various depths throughout the Gulf as observed in May, 1934 are shown in Fig. 4 and illustrate this fact. The general character of the phosphorus cycle may be supposed to be similar in all parts of the basin.

2. *Phosphate present as zoöplankton and nekton and not sampled by the water bottle may be ignored.* A large number of vertical zoöplankton hauls made throughout the years 1933–34 in all parts of the Gulf yielded an average catch of 40 cc. dry plankton per square meter of surface. It may be estimated from analyses made on such material that this would contain about 0.4 per cent of the total phosphorus in the water from which it was strained. Since the particulate organic phosphorus amounts to about ten times this quantity, it may be seen that the neglect of this fraction does not introduce a significant error.

3. *All synthesis of particulate or soluble organic phosphorus compounds from inorganic phosphate takes place in the upper layer.* This is justified by Clarke's measurements on the penetration of light into the Gulf of Maine and on determinations of the compensation point in photosynthesis by diatoms in bottle experiments at different depths (Clarke and Oster, 1934).

4. *All downward movement of phosphorus is due to the sinking of organisms (particulate organic phosphorus).* This is the only fraction affected by gravity. It is also the only fraction displaying a well-marked concentration gradient decreasing downward—a condition essential for downward dispersal by eddy conductivity.

5. *All upward movement of phosphorus is due to the transport of inorganic* PO_4 *by eddy conductivity.* The gradient of concentration of inorganic PO_4 increases downward and is well marked except in mid-

winter. The soluble organic phosphorus never develops a strong gradient in this direction. Since it is present in much smaller concentrations than is the inorganic phosphate, it may safely be ignored in considering vertical transport by eddy conductivity.

6. *All observed transformations in any layer are attributed to processes occurring in that layer, except so far as vertical transport must be postulated to account for the transformation.* This premise is introduced since without it a unique solution cannot be obtained. It implies that all values arrived at for vertical exchange are minimal.

7. *The portion of the exchange in which the cycle runs to completion is necessarily ignored. All values for the magnitude of the exchange are consequently minimal.*

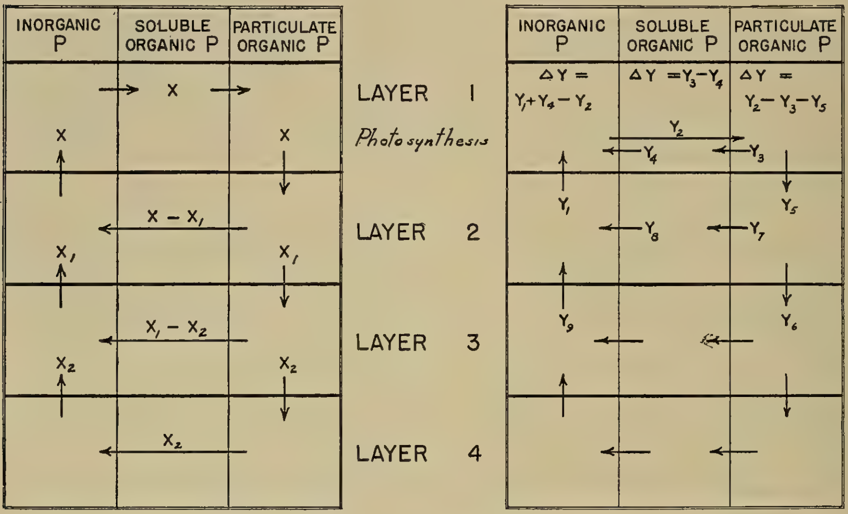
INORGANIC P
SOLUBLE ORGANIC P
PARTICULATE ORGANIC P
X
X
X
X − X,
X,
X,
X, − X₂
X₂
X₂
X₂
LAYER 1
Photosynthesis
LAYER 2
LAYER 3
LAYER 4
INORGANIC P
SOLUBLE ORGANIC P
PARTICULATE ORGANIC P
ΔY = Y₁+Y₄ − Y₂
ΔY = Y₃−Y₄
ΔY = Y₂− Y₃−Y₅
Y₂
Y₄
Y₃
Y₁
Y₅
Y₈
Y₇
Y₉
Y₆

FIG. 2. See text.

A consideration of this limitation may serve to make clear the general basis of the analysis. If we start with the system in a steady state and if, during the period between two sets of observations the cycle has proceeded without change in the relative velocity of the processes in any part, the distribution of the fractions of phosphate in all parts of the system will be the same at the end as it was in the beginning. This does not mean that no exchanges of phosphate between different parts of the system have taken place, rather that all exchanges are exactly compensated. While the system is in a steady state an unobservable quantity of phosphate is undergoing transformation from each stage in the cycle to the next stage. If the system is disturbed, as through seasonal changes in the physical conditions, then transformations of one sort may proceed more rapidly than those of another with the result that differences in the distribution of phosphorus are observed, and from these differences the magnitude and nature of the processes *which have caused* the differences may be deduced. The observations tell us nothing, however, of the basal level of activity on which the differences are superposed.[2]

Figure 2 illustrates the principle of the method. At any time there will be a basal level of activity represented by the transformation of an unobservable quantity of phosphorus, x, through each stage in the cycle. This quantity will have been transported upward from layer 2 into layer 1 to be synthesized into particulate organic form. If the system is to remain unchanged, the equivalent of this material must have sunk back into the deeper layers and been decomposed, passing through the soluble organic form, to exactly replace that which was transported upward. Portions of x, designated as x_1, x_2, etc. may sink to deeper layers before undergoing transformations from organic to inorganic form. The general conditions are that the quantity of x entering and leaving any part of the system shall be equal, that x move upward as inorganic phosphate (Postulate 5), and downward as filterable organic phosphorus (Postulate 4), that it represent synthesis of filterable organic phosphorus only in the upper layer, and that it represent a transformation of organic into inorganic phosphorus in any layer.

If the system is disturbed between observations, then changes in the quantity of phosphorus, ΔY, in any form and part of the system may be observed. These changes may be accounted for only by additional exchanges between the various parts of the system. The problem is to determine the minimal additional exchanges of this sort, Y_1, Y_2, Y_3 etc., which will account for the change in each part of the system in accordance with the postulates laid down above. The general conditions are that ΔY, the change in any fraction in any layer shall equal the difference in Y_1, Y_2, Y_3 etc., the amounts of phosphorus entering or leaving that fraction and layer during the period between observations. Furthermore, ΔY must be accounted for so far as possible by exchanges taking place within the layer in question (Postulate 6).

An attempted analysis of the changes in phosphorus distribution is presented in Tables VII, VIII and IX.

February to May (Table VII) represents the period in which the great spring flowering of phytoplankton occurs. During this period an amount of phosphorus equivalent to 9.1 per cent of the total disappears from the inorganic phosphate of the upper layer. Of this only 2.0 per cent can be accounted for as an increase in particulate and soluble organic phosphorus remaining in that layer. Seven and one-tenth per cent must have sunk to the deeper layers following its synthesis into organic matter. Only one-eighth of the phosphorus absorbed in photosynthesis has remained as particulate matter in the upper layer. This observation accords with the conclusion of Harvey (1934) that several times more vegetation is produced during the spring flowering of diatoms in the English Channel than is found there at the time of its maximum.

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[2] The method is analogous to the integration of a differential equation, the unobserved basal activity corresponding to the constant of integration. It is only in proportion as the system undergoes great seasonal fluctuation that the partial effects observed approach the total exchanges taking place. The method is applicable consequently particularly to studies made in high latitudes.

Table VII

Balance sheet of phosphorus exchanges February 26 to May 14, 1936. Numbers represent the change in the phosphorus fractions as percentages of total phosphorus in water column.

Depths		Inorganic phosphorus	Soluble organic phosphorus	Particulate organic phosphorus
0–60 meters	Photosynthesis	−9.1	→	+9.1
	Decomposition		+0.9 ←	−0.9
		0 ←	0	
	Exchange with layer below	0	0	−7.1
	Net change	−9.1	+0.9	+1.1
60–120 meters	Exchange with layer above	0	0	+7.1
	Decomposition		+4.7 ←	−4.7
		+1.4 ←	−1.4	
	Exchange with layer below	0	0	−2.1
	Net change	+1.4	+3.3	+0.3
120–180 meters	Exchange with layer above	0	0	+2.1
	Decomposition		+0.9 ←	−0.9
		+1.7 ←	−1.7	
	Exchange with layer below	+2.2	0	−1.4
	Net change	+3.9	−0.8	−0.2
180–240 meters	Exchange with layer above	−2.2	0	+1.4
	Decomposition	0	+1.1 ←	−1.1
	Net change	−2.2	+1.1	+0.3

To account for the increasing concentrations of inorganic phosphorus in the deeper layers after allowing for the greatest possible decomposition in situ at least 2.1 per cent must sink past the 120-meter level and 1.4 per cent past the 180-meter level. The phosphorus removed from inorganic form in the upper layer by photosynthesis is redistributed during the spring through considerable depths by the sinking of particulate matter. It is unnecessary to assume that any of the particulate matter sinks beyond the lower level before undergoing decomposition, though it is possible that this may be the case.

May to November (Table VIII) includes the greater part of the growing season. The important feature of this period is the appearance of large quantities of dissolved organic phosphorus at all depths. One-fifth of all the phosphorus in the water is in this form in November. This material can have been produced only by photosynthetic processes taking place in the upper layer. It must have been set free for the most part by decomposition of the particulate fraction in the layer in which it is observed (Postulate 5). In order to account for the

TABLE VIII

Balance sheet of phosphorus exchanges May 18, 1935 to November 8, 1935. Numbers represent the change in the phosphorus fractions as percentages of total phosphorus in water column.

Depths		Inorganic phosphorus	Soluble organic phosphorus	Particulate organic phosphorus
0–60 meters	Photosynthesis	−19.2	→	+19.2
	Decomposition		+3.5 ←	−3.5
		0 ←	0	
	Exchange with layer below	+18.6	0	−17.0
	Net change	−0.6	+3.5	−1.3
60–120 meters	Exchange with layer above	−18.6	0	+17.0
	Decomposition		+5.0 ←	−5.0
		0 ←	0	
	Exchange with layer below	+15.2	0	−12.3
	Net change	−3.4	+5.0	−0.3
120–180 meters	Exchange with layer above	−15.2	0	+12.3
	Decomposition		+4.8 ←	—4.8
		0 ←	0	
	Exchange with layer below	+10.8	0	−6.9
	Net change	−4.4	+4.8	+0.6
180–240 meters	Exchange with layer above	−10.8	0	+6.9
	Decomposition		+6.4 ←	−6.4
		0 ←	0	
	Net change	−10.8	+6.4	+0.5

quantities of dissolved organic phosphorus observed, a large vertical movement of inorganic phosphorus upward through all depths must be postulated as well as an equivalent sinking of organisms to the sites at which the soluble organic phosphate appears. Over 17 per cent of the total phosphorus in the water must pass through the zone of photosynthesis in the course of the six summer months. Since the account is balanced without supposing any phosphorus to pass from the organic back to the inorganic form, a process which must certainly be taking place, this figure may be far below that actually obtaining.

Table IX

Balance sheet of phosphorus exchanges November 8, 1935 to February 26, 1936. Numbers represent percentages of total phosphorus in entire water column.

Depths		Inorganic phosphorus	Soluble organic phosphorus	Particulate organic phosphorus
0–60 meters	Photosynthesis	0	→	0
	Decomposition		+0.6 ←	−0.6
		+4.2 ←	−4.2	
	Exchange with layer below	+4.2	0	−0.2
	Net change	+8.4	−3.6	−0.8
60–120 meters	Exchange with layer above	−4.2	0	+0.2
	Decomposition		0 ←	0
		+2.7 ←	−2.7	
	Exchange with layer below	+2.2	0	−0.2
	Net change	+0.7	−2.7	0
120–180 meters	Exchange with layer above	−2.2	0	+0.2
	Decomposition		0 ←	0
		+1.7 ←	−1.7	
	Exchange with layer below	0	0	−1.0
	Net change	−0.5	−1.7	−0.8
180–240 meters	Exchange with layer above	0	0	+1.0
	Decomposition		+1.7 ←	−1.7
		+7.5 ←	−7.5	
	Net change	+7.5	−5.8	−0.7

November to February (Table IX) is marked chiefly by the regeneration of inorganic phosphorus, which increases by 16 per cent of the total, and by the equalization of the concentration of this fraction throughout the water column. The table shows that this regeneration is made to a large extent at the expense of the soluble organic phosphorus, that the transformation takes place throughout the entire range of depths, though greatest near the bottom. The decomposition of organic phosphorus compounds in situ and the vertical transport of inorganic phosphate are about equally important in effecting the equalization of the concentration of the latter throughout the water column.

The Mechanism of Vertical Transport

The foregoing analysis indicates that very considerable exchanges of phosphorus take place between various depths of water. At the same time these exchanges appear to diminish in extent as the depth increases. Downward movement has been attributed to the sinking of particulate matter under the influence of gravity. There appears to be no difficulty in considering that in depths of a few hundred meters organized particles of the dimensions of diatoms would sink to the bottom before undergoing decomposition. If such were the case very large quantities of phosphorus would be withdrawn from the water during each growing season. This may be the case in shallow waters, but it does not appear to be happening in the western basin of the Gulf of Maine. If so the total phosphorus in the water should show a marked seasonal change. The situation is probably complicated by biological considerations. Harvey (1934) has presented evidence that the stock of phytoplankton is grazed down by zoöplankton during the summer. This conclusion suggests that the zoöplankton are important active agents in converting particulate organic phosphorus into its decomposition products. Since these animals, and particularly the copepods, make extensive diurnal vertical migrations, and since some time must elapse between the taking of food near the surface and its elimination as waste products, they provide an agency for a limited vertical transport of organic material. From this viewpoint the zoöplankton become an important agency in maintaining the fertility of the water for phytoplankton, since they hasten the conversion of bound nutrients into inorganic form and prevent these nutrients from becoming unavailable by the sinking of particulate matter to great depths or to the bottom.

The vertical transport of inorganic phosphate is simpler since it can be effected only by the mixing of the water. It is pertinent to inquire whether the conditions are such as to permit of the amounts of transport deduced during the various periods of observation. The amount of a constituent, Q, passing through unit horizontal surface in unit time depends upon the gradient of concentration of the constituent

dc/ds and the coefficient of eddy conductivity, A (Austausch coefficient).

$$Q = A dc/ds$$

The coefficient of eddy conductivity, A, represents the volume of water exchanged through each horizontal unit surface in unit time.

The gradient of phosphate concentration observed in February, May and November is shown in Fig. 3. Between May and November, when large vertical movements have been deduced, a well-marked gradient exists particularly in the upper layers, as is required for such movements. In February this gradient has disappeared, and the concentration of phosphate is equal at all depths down to 150 meters. No amount of mixing can effect a change in its vertical distribution. Since this condition must exist during a considerable portion of the winter, it is not surprising that the vertical transports *deduced from our data* between November and May are smaller than those observed in the summer.

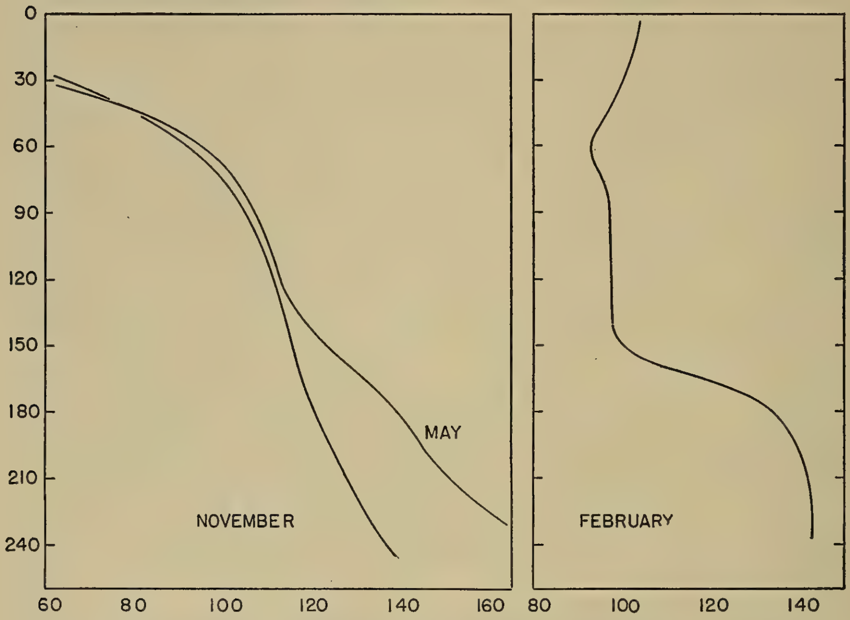

FIG. 3. Distribution of inorganic phosphate concentration with depth in November, 1935 and February and May, 1936 at standard station in the western basin of the Gulf of Maine. Depths in meters measured downward along the ordinate; concentrations in milligrams PO_4 per cubic meter along the abscissa.

A knowledge of the coefficient of eddy conductivity, A, is the key to understanding the nutritive conditions in deep bodies of water.

Methods of estimating its value are so indirect that little is known of its magnitude under any circumstances. Although our data are admittedly very unprecise and yield only minimal values for the exchange, it is nevertheless of some interest to use it in estimating the coefficient required to account for the transport of phosphate in the Gulf of Maine. The gradient of phosphate concentration is sufficiently uniform throughout the period May–November to permit a single value to be taken at any level as representative of the entire period. This is not the case during the remainder of the year. We have calculated the value of A for the boundary of each of the layers, using the data presented in Table VIII for estimating Q and the slopes of the curves in Fig. 3 for dc/ds. The result is shown in Table X. The values of A are minimal and are less reliable at the greater depths. The values obtained are not unreasonable. Seiwell considered A to equal 2 C.G.S. units in the thermocline of the tropical Atlantic, whereas values increasing to 50 C.G.S. units have been obtained in various waters (Seiwell, 1935).

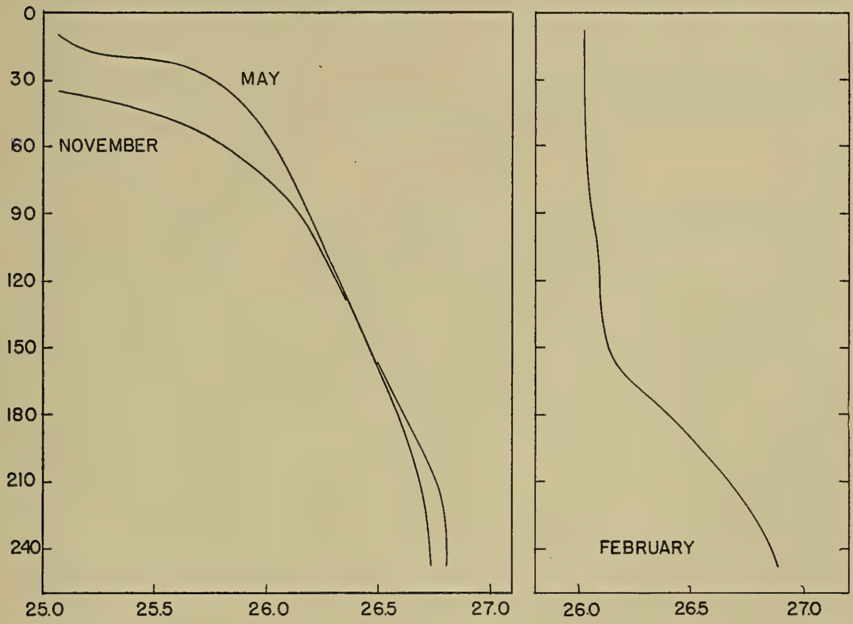

FIG. 4. Distribution of density, σ_t, with depth in November, 1935, and February and May, 1936, at standard station in the western basin of the Gulf of Maine. Depths in meters measured downward along the ordinate; density, σ_t, measured along the abscissa.

The value of the coefficient of eddy conductivity depends upon the forces responsible for mixing and varies inversely with the stability, $\frac{d\sigma_t}{ds}$ of the water. If K represent the mixing forces,

$$K = A \times \frac{d\sigma_t}{ds}$$

Seiwell considers that $K = 4.73 \times 10^{-4}$ in the thermocline of the North Atlantic. Figure 7 shows the distribution of density, σ_t, with depth at the station in the Gulf of Maine in May, 1935 and in November. From these curves the representative values of $\frac{d\sigma_t}{ds}$ entered in Table X are taken. The values of K given by multiplying $\frac{d\sigma_t}{ds}$ by A are of the order obtained by Seiwell. It is concluded that the vertical transport of inorganic phosphate deduced from the seasonal change in the distribution of the various fractions of phosphorus compounds does not require unreasonable assumptions concerning the magnitude of the eddy conductivity.

TABLE X

Estimation of coefficient of eddy conductivity in Gulf of Maine, May to November, 1935.

Depths	Q		dc/ds	A	$d\sigma_t/ds$	$K = A \times \frac{d\sigma_t}{ds}$
meters	*per cent in 3 months*	$\frac{mg.}{cm.^2 \times sec.}$	$\frac{mg.}{ml.\ cm.}$	$\frac{ml.}{cm. \times sec.}$	$\frac{grams}{ml. \times cm.}$	
60	>18	$>3.5 \times 10^{-8}$	6.6×10^{-9}	>5.2	1.0×10^{-4}	$>5.2 \times 10^{-4}$
120	>15	$>2.4 \times 10^{-8}$	2.0×10^{-9}	>12.0	0.5×10^{-4}	$>6.0 \times 10^{-4}$
180	>10	$>2.0 \times 10^{-8}$	3.0×10^{-9}	>6.6	0.4×10^{-4}	$>2.6 \times 10^{-4}$

SUMMARY

1. Methods are described for the determination of the phosphorus present in particulate form and of the total phosphorus in a sample of sea water.

2. The distribution of phosphorus present as inorganic phosphate, as dissolved organic compounds, and as particulate matter (detritus and microörganisms) has been determined at all depths throughout the year at a station in the western part of the Gulf of Maine.

3. In late winter over 90 per cent of the phosphorus is in inorganic form and three-quarters of the remainder is present as soluble organic compounds.

4. In the spring—February to May, inorganic phosphorus is converted to organic form by photosynthesis in the upper layer of water. Most of this fraction sinks to considerable depths before undergoing decomposition.

5. During the summer—May to November, large quantities of dissolved organic phosphorus appear at all depths, indicating a very considerable transport of inorganic phosphate from deep water to the surface and the sinking of an equivalent amount of phosphorus in particulate form to the depths in which organic compounds are liberated by decomposition. Decomposition appears to take place throughout the water column.

6. During the winter—November to February, the organic phosphorus compounds are converted to inorganic phosphate. This and vertical mixing of preformed phosphate are about equally important in bringing about the equalization of phosphate concentrations throughout the depth of water.

7. A method is described for analyzing quantitatively the factors producing a seasonal change in the distribution of a compound such as phosphorus. It is shown that the vertical transport of material within the water mass demanded by such an analysis may be accounted for reasonably by the hydrographic conditions obtaining.

8. Values of the coefficient of eddy conductivity at several depths are obtained.

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GROWTH AND VARIABILITY IN DAPHNIA PULEX

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INTRODUCTION

The aim of this study is to determine the number of pre-adult instars, growth, relative growth, and variability of individually reared female *Daphnia pulex* De Geer.

Numerous studies on growth of Cladocera have been made. Most of them are based on size-frequency distributions in natural populations. Some are based on experiments with individually reared animals. These studies have been carried on largely by Woltereck and his students, especially Rammner. Size-frequency distribution methods are inadequate for growth determinations as will be brought out more fully later. Previous studies carried out by means of individually reared animals deal with small numbers of organisms, a short part of the possible life span of an individual, or both. Recently Banta and his co-workers have carried out intensive studies on large numbers of individually reared *Daphnia longispina* which lived for long periods. In the present study a large number of animals has been observed for a relatively long time.

PROCEDURE

Essentially the same procedure was used as that employed by Anderson (1932) for *Daphnia magna*. Individual female *Daphnia pulex* of a single clone were isolated within eight hours after their release from their mothers and reared in manure-soil medium (Banta, 1921). Each individual was placed in a separate glass vial containing 20–25 cc. of the medium. Semi-weekly one-half of the total volume was replaced with fresh medium. The animals were kept at room temperature (15°–22° C.).

At the time of isolation and daily thereafter, each individual animal was placed in a watch glass together with a few drops of culture medium. Just enough saturated chloretone solution was added to bring about cessation of movement. Measurements as shown in Fig. 1 were made by means of an ocular micrometer. The animals were never in the chloretone solution for more than five minutes at any one time.

Immediately before the daily mensurations note was taken of cast carapaces and the number of young released. These were removed at the time of their discovery.

RESULTS AND DISCUSSION

Longevity

Some 82 animals were observed in these experiments. Figure 2 is a survival curve. Fifty-one animals lived for twenty instars and of these, 3 continued to the twenty-fifth instar. Bourguillaut de Kerherve (1926) observed two *Daphnia magna* for nineteen instars. Each one released nineteen clutches of young and since not more than one clutch of young is released each instar and at least five instars precede the release of the first clutch of young, these two *Daphnia magna* lived for twenty-four instars or more. He cites 4 other individuals which must have reached the twenty-second, the thirteenth, the tenth, and the sixth instar, respectively. Anderson (1932) observed some 30 *Daphnia magna* for fourteen instars, 32 for thirteen instars, and others for a smaller number of instars, all from the time of release from the mothers. Rammner (1928) observed one *Scapholeberis mucronata* for seventeen instars and another for nine. Rammner (1929) cites others that have been observed for shorter times. Ingle, Wood, and Banta (1937) have observed individually reared *Daphnia longispina* for over twenty-five instars.

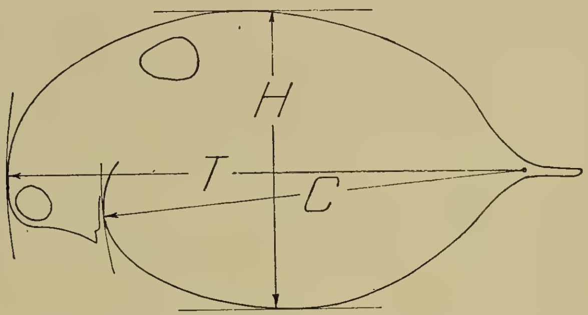

FIG. 1. Diagram showing method of making measurements. *T*, total length, longest dimension of animal exclusive of spine. *C*, carapace length, longest dimension of the carapace exclusive of spine. *H*, height, the shortest distance between two lines tangent to the carapace and parallel to the line of *T*. This measure of height is affected very little by the number of young in the brood chamber.

Of the 82 animals observed in the present study, 71 were primiparous during the fifth instar and 9 during the sixth, while the remaining 2 died during the second and third instars. The number of pre-adult instars is therefore variable in this species as well as in others (Anderson, 1932). The minimum number of pre-adult instars for *Daphnia pulex* is probably four.

Of the 71 animals primiparous during the fifth instar, 47 lived through the twentieth instar. The data from these 47, summarized in Table I, are the basis for the growth studies which follow.

Absolute Growth

Figure 3 is a group of growth curves in terms of total length, carapace length, and height. The curves are similar in shape. The point of inflection in each curve comes immediately before the instar during which the animals are primiparous. Attempts have been made to fit the Robertson and Gompertz equations to the curves. In neither instance was the result judged satisfactory. The time unit used in this work is the instar. Adult instars have been considered as equivalent physiological time units (Anderson, 1933). At room temperature, each of the first three pre-adult instars lasts approximately one day, the fourth or last pre-adult instar one and a half days, the first adult instar about two days, and each subsequent instar becomes increasingly longer. The use of the instar as a time unit

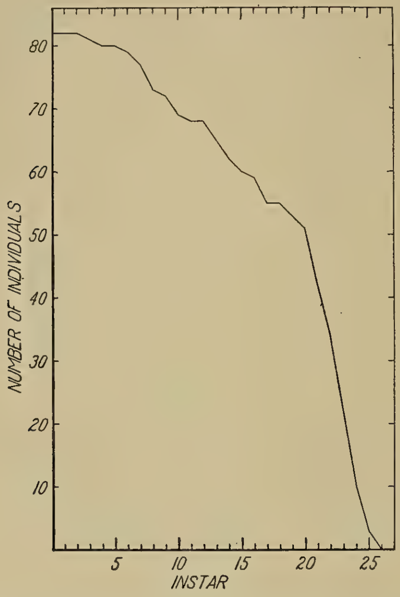

FIG. 2. Survival curve for the eighty-two animals observed in these experiments.

may be the cause of unsatisfactory results in attempting to fit the equations to the present data.

When the logarithms of total length are plotted against time in instars, a curve is obtained which may be broken up into three distinct segments each of which approximates a straight line. The first segment takes in the first five instars, the second segment includes the next five, and the third segment embraces instars eleven to twenty.

Figure 4 shows the growth increments in terms of total length, carapace length, and height. The increments increase up to the fourth instar then gradually decrease until the eleventh instar, after which they remain much the same.

It is of interest to determine to what degree such characteristics of the growth process as initial size, final size, duration of growth, and initial velocity of growth are interdependent. As measures of these quantities, total length in the first instar (a), total length in the twentieth instar (A), the number of instars required to attain a length of approximately $0.8A$ (t), and the increment between the first and second instars (i), respectively, were employed. For these the following coefficients of correlation were obtained:

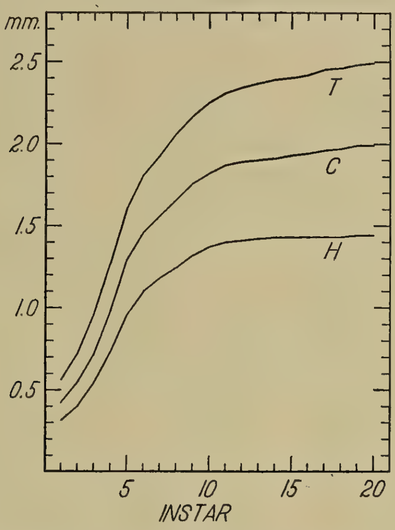

FIG. 3. Growth curves based on data from the 47 animals which were primiparous in the fifth instar and lived for twenty instars or more. T, total length; C, carapace length; H, height.

$$
\begin{aligned}
r_{aA} &= 0.2309 \pm 0.0931\\
r_{ai} &= -0.4481 \pm 0.0792\\
r_{Ai} &= -0.1483 \pm 0.0963\\
r_{at} &= 0.1641 \pm 0.0957\\
r_{At} &= -0.3893 \pm 0.0835
\end{aligned}
$$

It is apparent that only r_{ai} and r_{At} are significantly different from zero. There is thus an indication of an inverse relationship between initial size and initial velocity of growth, and between duration of growth and final size. On the other hand, there is no evident relationship between initial and final size, between initial size and duration of growth, or between initial velocity and final size.

Similar coefficients of correlation have been computed for *Daphnia longispina* by Wood and Banta (1936). These authors found that with unlimited food early growth tends to be inversely related to size at the time of release, but that when the quantity of food is limited these factors vary independently. They found also that initially larger animals tend to become larger adults, which is contrary to the results obtained from our data.

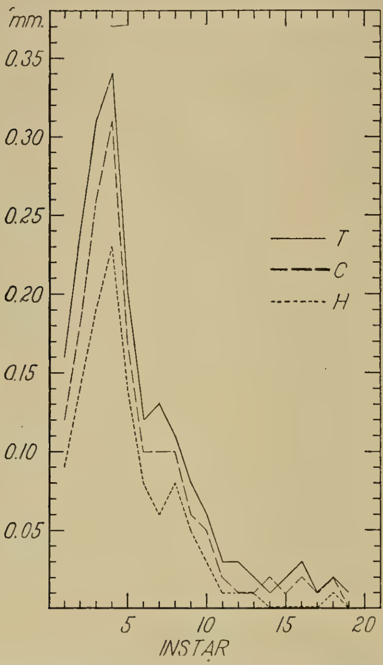

FIG. 4. Growth increment curves for the same animals as in Fig. 3. *T*, total length; *C*, carapace length; *H*, height.

A negative correlation between initial size and initial growth rate has been found to occur also in certain mammals. Thus it has been observed that in man (Hammett, 1918) and in the cat (Hall and Pierce, 1934) the percentage increase in body weight in the period immediately following birth is inversely proportional to birth weight.

Hammett (1918) interprets these results as indicating that differences in birth weight correspond to differences in physiological age at the time of birth, the physiologically younger individuals having the greater percentage growth rate.

In multiparous mammals the situation is often complicated by variation in litter size and attendant differences in food supply of the sucklings. Crozier and Enzmann (1935) showed that in inbred albino mice there is a hyperbolic relationship between birth weight and litter size, also that individuals in large litters grow more slowly at first than those in small litters, due to the decreased quantity of available milk per animal. After the suckling period, however, the growth of the smaller individuals is accelerated so that they eventually catch up with the others, and all attain the same adult weight regardless of birth weight.

In order to eliminate differences in nutrition, Kopeć (1932), working with a non-inbred stock of mice, reduced all litters to the same size. He found that birth weight varied inversely with litter size, and that individuals born in large litters exhibited a higher initial percentage growth rate than those born in small litters. When the data were seriated on the basis of birth weight without regard to litter size, however, there was found to be no correlation. Kopeć, who regards the different size groups in the second case as representing different genetic types, concluded that genetic differences in birth weight have no effect on subsequent growth, as do environmental differences. The validity of this argument seems open to question. It would be interesting in this connection to determine whether or not the same results occur with an inbred stock, also to investigate similarly the effects of brood size in Cladocera.

The occurrence of a significant negative correlation between duration of growth and final size in the present case appears to be in line with the results of McCay, Crowell, and Maynard (1935). These authors observed that in white rats, individuals whose growth period has been prolonged by partial starvation attain on adequate feeding a final size lower than that of controls given sufficient food throughout. Ingle, Wood, and Banta (1937), however, in a similar experiment on *Daphnia longispina*, found practically no difference in final size between the experimental animals and the controls. Also Merrell (1931) found that in rabbits there is no significant correlation between the time required to reach one-half the adult weight and the adult weight attained. On the other hand, Merrell's observation that initial growth rate is not associated with adult weight is in agreement with our results.

Reproduction

Figure 5 gives the average number of living young released during each instar. The number increases until the tenth instar followed by a gradual decrease. The period of increase corresponds to the time when the growth is falling most rapidly. The period of decrease corresponds to the time when growth has practically ceased.

The number of young released by a daphnid during any one instar has been used as an index of its condition (Anderson, 1933). The data available in the present study may serve as a test for this assumption. The young released in any instar develop from eggs produced during the instar before. Consequently, the coefficient of correlation must be computed from the growth increment with respect to total length for instar five and the number of young released in instar six, etc. The coefficients for the fifth, sixth, and tenth instars were determined. These are .690 ± .025, .649 ± .028, and .645 ± .030, respectively. Hence one may conclude that the number of young produced may serve as a valid index of the condition of a daphnid.

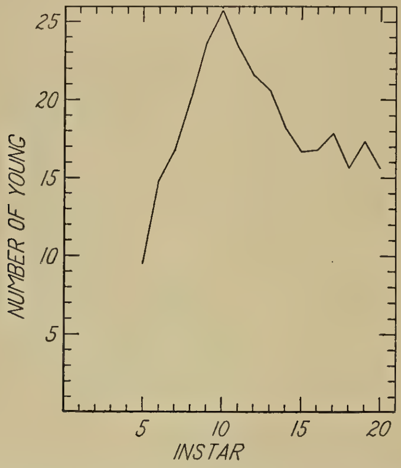

FIG. 5. Curve of the average number of living young released during each instar by the same animals as in Fig. 3.

Relative Growth

Relative growth studies show certain characteristics of this species. In Fig. 6 the logarithms of carapace length were plotted against those of total length, those of height against those of total length, and those of carapace length against those of height. The relation of carapace length to total length can be expressed satisfactorily on this *log log* plot by three straight lines. The first of these can be drawn through the points for the pre-adult instars (1–4), the second through the points for the next eight instars (5–12) and the third through the last eight instars (13–20) represented. The relations between height and total length are similar to those between carapace length and total length. The relations between carapace length and height are different. Two lines may be used, one for the first ten instars and a second for the last ten (11–20). These linear relations can be expressed as

$$y = bx^{\alpha},$$

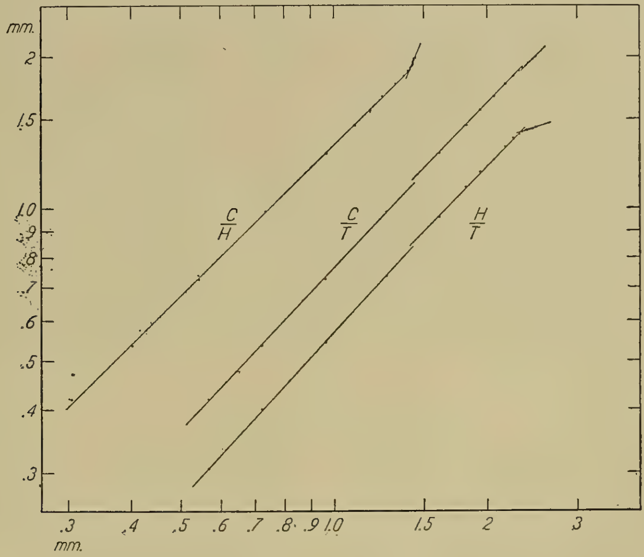

FIG. 6. Double logarithmic plots of the relations between carapace length and total length C/T, height and total length H/T, and carapace length and height C/H during each instar for the same animals as in Fig. 3.

where b is a constant—the initial growth index, α is the equilibrium constant, and x and y are values of two parts (Huxley and Teissier, 1936). The values of the constants are given in Table II. These values were calculated by the least squares method.

The relationships shown above are much the same as those secured for *Daphnia magna* by Anderson (1932). No very marked change in proportions occurs as may be seen in Fig. 7. The marked change in relative growth that occurs at the twelfth and thirteenth instars as indicated in Fig. 6 can hardly be recognized by inspecting Fig. 7 alone. The lines representing the thirteenth to the twentieth instars in Fig. 6 are very short since growth is very much retarded during this period.

TABLE I

Mean values of total length, carapace length, and height and their probable errors in millimeters for each instar. Also the standard deviation (σ) and the coefficient of variation (V) for total length.

Instar	Total length	Standard deviation	Coefficient of variation	Carapace length	Height
1	0.5665±0.0034	0.0347±0.0024	6.13±0.43	0.4194±0.0027	0.3056±0.0021
2	0.7214±0.0038	0.0389±0.0027	5.39±0.37	0.5361±0.0034	0.4015±0.0027
3	0.9609±0.0056	0.0568±0.0040	5.92±0.41	0.7244±0.0048	0.5433±0.0039
4	1.2653±0.0092	0.0934±0.0065	7.38±0.51	0.9848±0.0078	0.7346±0.0054
5	1.6079±0.0095	0.0964±0.0067	6.00±0.42	1.2850±0.0082	0.9597±0.0059
6	1.8134±0.0106	0.1081±0.0075	5.96±0.41	1.4572±0.0097	1.1015±0.0084
7	1.9332±0.0117	0.1196±0.0083	6.19±0.43	1.5591±0.0110	1.1778±0.0094
8	2.0571±0.0130	0.1316±0.0092	6.40±0.45	1.6615±0.0111	1.2445±0.0096
9	2.1673±0.0126	0.1275±0.0089	5.88±0.41	1.7587±0.0110	1.3213±0.0090
10	2.2466±0.0116	0.1174±0.0082	5.23±0.36	1.8200±0.0103	1.3738±0.0088
11	2.3061±0.0108	0.1095±0.0076	4.75±0.33	1.8653±0.0093	1.3976±0.0083
12	2.3401±0.0099	0.1007±0.0070	4.31±0.30	1.8891±0.0088	1.4131±0.0071
13	2.3705±0.0092	0.0939±0.0065	3.96±0.28	1.9034±0.0081	1.4227±0.0066
14	2.3853±0.0095	0.0962±0.0067	4.03±0.28	1.9135±0.0081	1.4262±0.0071
15	2.4032±0.0092	0.0935±0.0065	3.89±0.27	1.9326±0.0075	1.4274±0.0065
16	2.4223±0.0085	0.0866±0.0060	3.58±0.25	1.9433±0.0072	1.4274±0.0053
17	2.4461±0.0072	0.0736±0.0051	3.01±0.21	1.9594±0.0055	1.4340±0.0047
18	2.4604±0.0072	0.0730±0.0051	2.97±0.21	1.9689±0.0060	1.4340±0.0046
19	2.4835±0.0072	0.0730±0.0051	2.94±0.20	1.9862±0.0061	1.4435±0.0049
20	2.4890±0.0072	0.0731±0.0051	2.94±0.20	1.9928±0.0061	1.4429±0.0047

Variability in Body Size

Variability in body size and its fluctuations during growth have been studied in other forms by a number of investigators. The present data are particularly suitable for such a study, since it may safely be assumed that the animals are genetically homogeneous and that genetic differences are thus eliminated as a source of variation.

TABLE II

Values of the constants b and α for the data shown in Fig. 7.

Relation	Instars	b	α
Carapace length y.............. Total length x..............	1–4	0.762	1.06
	5–12	0.790	1.03
	13–20	0.814	0.985
Height y.............. Total length x..............	1–4	0.417	1.09
	5–12	0.530	1.03
	13–20	12.49	0.315
Carapace length y.............. Height x..............	1–10	1.333	0.99
	11–20	0.848	2.34

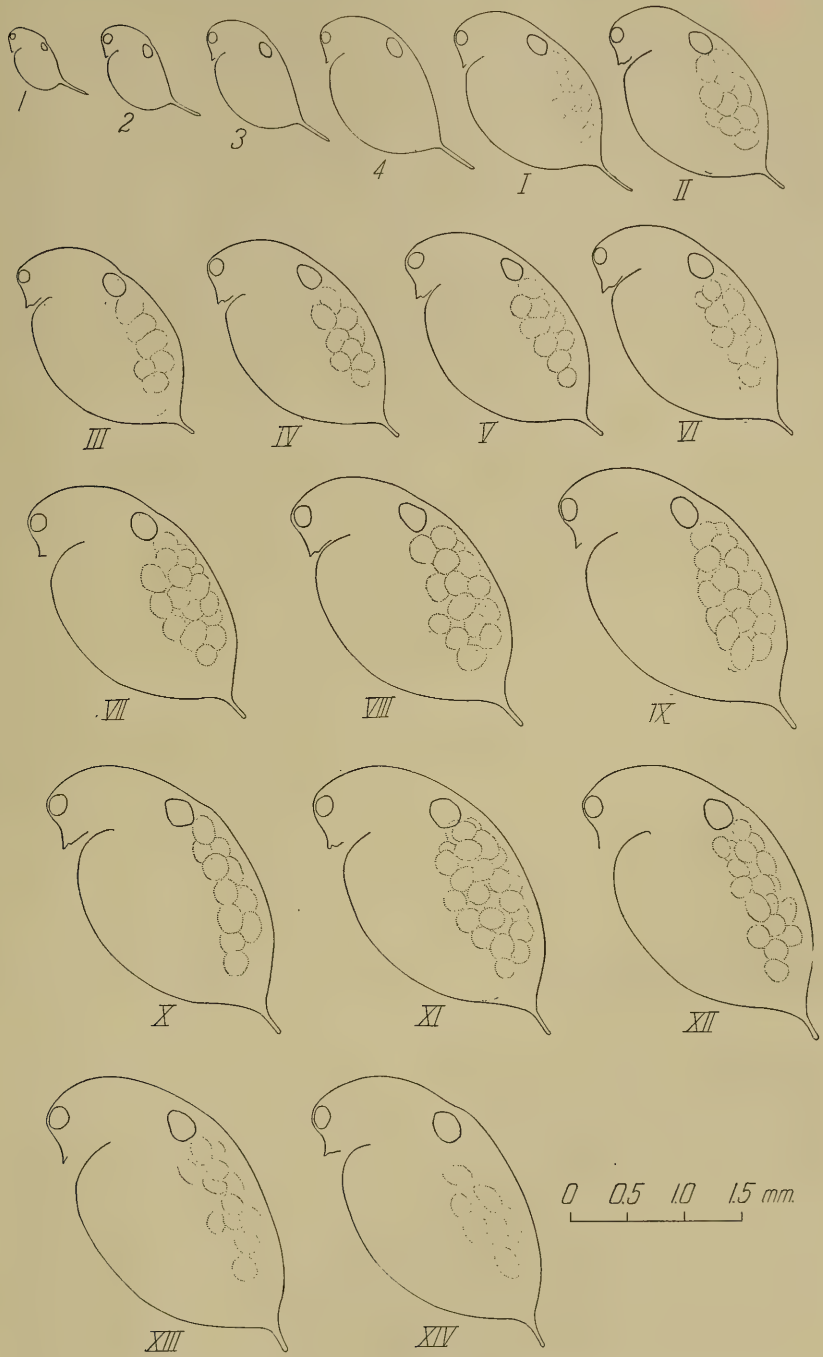
1
2
3
4
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
0 0.5 1.0 1.5 mm.

FIG. 7. Camera lucida outline drawings of a single animal for each of the first eighteen instars. Arabic numerals designate pre-adult instars; Roman numerals—adult instars.

Total length was employed as the best available measure of body size. Its use in this way is justified by the fact that there is apparently very little change in the shape of the body during growth (see the preceding section). The standard deviations and coefficients of variation for total length are given in Table I and are plotted against instar number in Fig. 8. It may be noted that both these quantities tend to increase at first, but that after the eighth instar they fall off rather steadily until the seventeenth instar, after which they remain approximately constant. Hence as the growth process reaches completion, body size becomes less variable both absolutely and relatively.

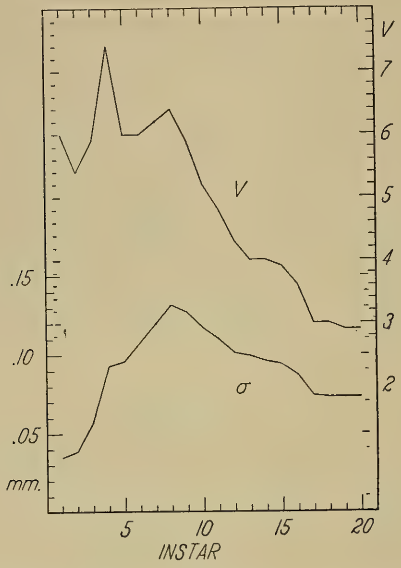

FIG. 8. Standard deviation and coefficient of variation of total length in relation to instar number.

A comparison of the curve for the coefficient of variation with the increment curve (Fig. 4) suggests that these two quantities are correlated. When the coefficient of variation is plotted against the logarithm of the increment (Fig. 9), the points tend to group themselves along a straight line, indicating that there is roughly a linear relationship between the relative variability and the logarithm of the growth rate. Whether or not any general significance can be attached to the precise character of the relationship as indicated here is open to question; however, there is no doubt that a distinct relationship exists.

These observations are essentially in agreement with those of previous investigators. A trend in the coefficient of variation similar to that obtained here has been found to occur in the case of both stature and body weight in man by several workers, including Bowditch (1877), Thoma (1882), Porter (1894), Boas (1897), Boas and Wissler (1904), and others. A summary and discussion of the data of Bowditch, Boas, and Boas and Wissler is given by Thompson (1917, pp. 78–80). Essentially the same trend has been observed for body weight in the albino rat by Jackson (1913), King (1915), and Hanson and Heys (1927), in the Norway rat by King (1923), and in the cat by Hall and Pierce (1934). King (1918, 1919) has shown also that the trend persists in albino rats inbred for as many as twenty-five generations, although the coefficient of variability in such rats is consistently lower than that in non-inbred animals of the same average body weight. Weymouth and McMillin (1930) have found that in the Pacific razor clam, *Siliqua patula*, relative variability in shell length, as measured by the ratio interdecile range: median, decreases steadily with increasing length.

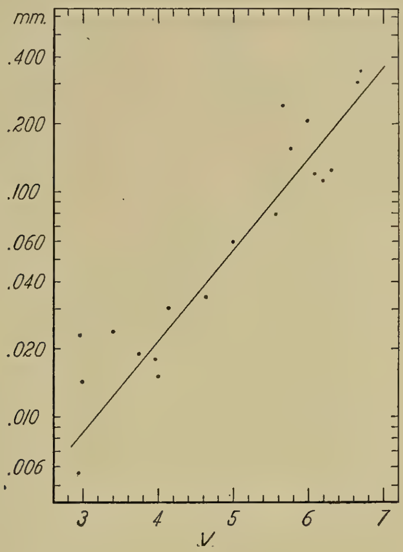

FIG. 9. Graph of the coefficient of variation of total length plotted against the logarithm of the increment. The values of the coefficient employed are averages of successive pairs in Table I.

Several of these investigators have remarked also the existence of a correlation between the coefficient of variation and the growth rate (*cf.* Thoma, 1882; Porter, 1894; Boas, 1897; Boas and Wissler, 1904; King, 1915, 1923). Jackson (1913), on the other hand, states that his data show no evident correlation between these quantities.

Thompson (1917) computed from the data of Boas the coefficients of variation for annual increments in human stature between the ages of five and eighteen, and found that these increased steadily with age. He attributes this to increasing differences in phase of growth among individuals of the group as growth proceeds. Analogous coefficients of variation computed for the present data fluctuate somewhat irregularly, but show on the whole a marked upward trend. In fact, from the thirteenth instar on, the values of the coefficients are considerably higher than 100 per cent. These values, however, are of doubtful significance, since the mean increments for these instars are small. When the value of the mean is close to zero, as Philiptschenko (1927) has pointed out, the coefficient of variation becomes unreliable as a measure of variability. As a matter of fact, one would expect that as the growth process comes to a close, the differences in phase of growth would tend to decrease (i.e. the increments for all individuals would ultimately become zero), and that variability in growth rate would correspondingly decrease.

The similarity of results obtained in the cases thus far investigated suggests that the relationships described above are of rather general occurrence, and that it may be worthwhile to seek a general explanation for them. In attempting to find such an explanation, it is necessary first of all to consider the sources of the observed variability, and the relative importance of each. There are in all four possible sources, namely errors in measurement, genetic differences, environmental differences, and the fundamental nature of the growth process itself.

It does not seem likely that the observed trend in variability can be accounted for to any significant degree on the basis of error in measurement. Assuming that the distribution of errors is Gaussian, we should expect that in measurements of length or weight, the absolute error, as indicated by the standard deviation, would remain constant or would, in some cases, increase with increasing magnitude of the object being measured. In the former case, the relative variability would decrease with growth; in the latter its behavior would vary, depending on the particular conditions. There is, however, no evident feature of such observational error which would account for a trend in the standard deviation such as that shown in Fig. 8, nor for the existence of a relationship between the coefficient of variability and the growth rate.

Some information concerning the rôle of genetic differences can be obtained from the data of King (1919) on the growth of inbred and non-inbred albino rats raised under identical laboratory conditions. Although the trend in the coefficient of variation is essentially the same in both stocks, the degree of correlation between the coefficient and the growth rate, as indicated by the scatter of points on a graph, is much higher for the inbred rats than for the others. This may be interpreted to mean that variability due to genetic factors tends to be largely independent of the velocity of growth.

On the other hand, variability due to environmental differences would be expected to vary with the growth rate, for an individual in a state of rapid growth is relatively highly sensitive to the action of environmental agents, and a given fluctuation from the norm would produce a greater deviation in growth than it would in an individual with a low growth rate. This has been recognized by Plunkett (1932) in connection with developmental processes in general. He points out that as such a process asymptotically nears completion, the organisms tend to become more stable and less sensitive to the effects of environmental factors such as, for example, temperature. Hence the variability with respect to the particular character involved will decrease. However, he erroneously assumes that the converse is also true, namely that if one group of organisms is less variable in a certain respect than another group, it is therefore nearer to the completion of the underlying developmental process. This may or may not be generally true, but it clearly requires further demonstration.

It has generally been assumed that variability in growing organisms, aside from that introduced through errors in measurement, must be due to either genetic or environmental differences. According to a recent theory proposed by Rahn (1932), however, such variability arises at least in part from the physico-chemical nature of the growth process itself. The theory is briefly as follows. The division of a cell must be preceded by the doubling of all its genes. Let us consider a particular gene in each of a number of unicellular organisms which are identical with respect to genetic and environmental factors. If we assume that the doubling of this gene in the various cells conforms to the law of mass action, then it follows that it will not double simultaneously in all the cells, but will do so over a definite time interval. If the same is true of each of the remaining genes, there will be a variability in division time of the cells.

Rahn has developed a simple mathematical formulation of the theory which he has applied to data on the division rate of bacteria. For multicellular forms, he has succeeded in analyzing only the case of a hypothetical organism in which all the cells are alike and in which the percentage rate of cell division is constant. He has shown that such organisms will vary with respect to the time required for the completion of a given number of cell generations. Moreover, as the number of generations increases, the frequency curves become flatter while the relative spread of variation decreases. In other words, there will be an increasing standard deviation and a decreasing coefficient of variation. If the theory is valid, it would follow that variability in cell number (and in body size insofar as it depends on cell number) in successive time intervals should behave in a similar fashion.

Obviously these conclusions are not directly applicable to growth in actual multicellular organisms for a number of reasons. First of all, such growth is generally characterized by a decreasing percentage growth rate; secondly, it involves increase in cell size, as well as in cell number; and thirdly, the average reproductive rates of different types of cells are not equal. Before the conformity of the theory with the observed results can be tested, the former must receive a much more elaborate mathematical formulation, and a theoretical analysis must be made in which these factors are taken into account.

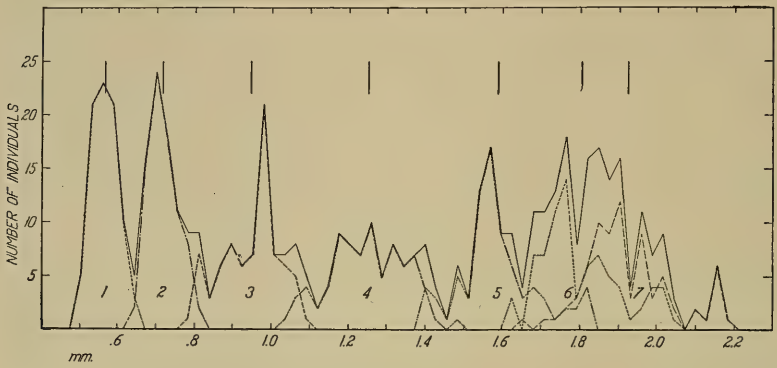

FIG. 10. Size frequency distributions during the first seven instars for all eighty-two animals observed. Broken lines designate the individual instars. The solid line is a composite curve for all instars. The vertical bars near the upper edge of the figure represent the mean total lengths for each of the first seven instars.

It may therefore be tentatively concluded that the observed trend in variability in body size with growth and its relation to growth rate are explicable largely in terms of the action of environmental factors, and perhaps also in part in terms of Rahn's theory.

Size-frequency Distribution

Most studies of growth in Cladocera have been made by analysis of size-frequency distributions in natural populations. These have been of two types (see Woltereck, 1929). One consists of plotting the number of individuals in a size class against size. In the graph that results a number of size modes appear which are taken as representative of the growth stages or instars. A second type consists of plotting the value for one dimension of an individual against another dimension of the same individual for each animal in a population. Usually the points fall into groups which are more or less distinct. Each group of points is taken as representative of a single instar.

No one so far as we are aware has attempted a comparison between the results secured by these methods and those secured by observations on individually reared animals. To make such a comparison, Figs. 10 and 11 were constructed. First the values for total length frequency during the first instar for all 82 animals observed were plotted. Then those for the second instar and so on to the seventh instar. After that a composite curve for all the values was plotted. The separate instar curves are shown in broken lines and the composite curve in a solid line in Fig. 10. The vertical lines in the upper part of the figure represent the mean total lengths for each instar up to and including the seventh. It is obvious that each mode in the composite curve does not represent the mean total length for an instar. The first three distinct modes are near the mean values. No distinct mode is apparent for the fourth instar. The mean value for the fifth

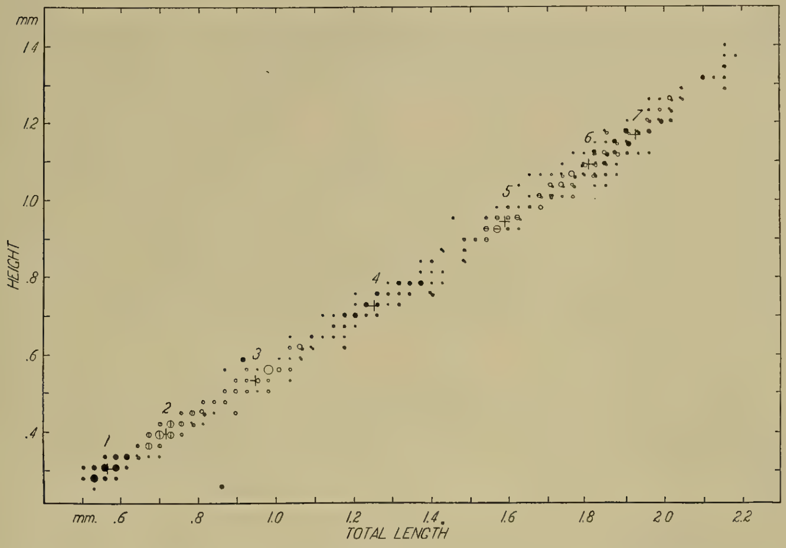

FIG. 11. Size frequency distributions in two dimensions during the first seven instars for all 82 animals observed. Each instar is represented by a different type of circle. The area of each circle is directly proportional to the number of animals of the particular dimensions represented by the circle. The crosses are the mean values for each of the first seven instars.

instar falls near a mode but the means of the sixth and seventh instars fall midway between modes. Further, considerable overlapping occurs between the values for various instars. The greatest value for the first instar is larger than the smallest for the second. Similar are the cases for the second and third instars, the third and fourth, and the fourth and fifth. Some of the values for the fifth are greater than the lowest for the seventh instar. This size-frequency method may be of some use for the pre-adult instars, but certainly not for adult instars.

Figure 11 was constructed by plotting the values of height against that of total length for all 82 animals for the first instar, followed by those of the second instar, and so on through the seventh. The area of each circle is directly proportional to the number of individuals represented. Where values for two or more instars fall on a point separate circles are used for each instar. The mean height and the mean total length for each instar are represented by crosses. This method differs from that described by Woltereck (1929) in that each individual was represented by a point on the graph while in the present case if more than one individual is of the same size the circle at point on the graph is larger and its area proportional to the number of individuals represented.

In analyzing Fig. 11, it will be noted that a band of circles is secured but no distinct groups. The circles of the greatest area may be considered to represent the instars. For the first three instars, the circles of greatest area fall near the mean values for the instars. No conspicuously large circle is found to represent the fourth instar. The cross for the fifth instar falls near a large circle but the crosses for the sixth and seventh instars do not. In most plots of natural populations (Woltereck, 1929) the points scatter more widely, i.e., they form a wider band than is the case in Fig. 11. Here again it is obvious that size-frequency distribution does not lend itself to a satisfactory study of growth.

Studies on growth by means of individually reared animals are advantageous over the above methods in several respects. The individual life histories are fully known. Genetic constancy can be maintained since diploid parthenogenesis is the only means of reproduction. Selection of young from one clone insures genetic constancy except for mutations. The environment can be maintained fairly constant. By natural population analysis methods the individual histories are unknown, the genetic constancy cannot be controlled, and the environment is subject to considerable fluctuation.

SUMMARY

Eighty-two individually reared female *Daphnia pulex* were observed from the time they were released from the brood chamber of their mothers until they died. Measurements of total length, carapace length, and height were made daily on each animal. The number of young released during each instar was recorded.

Seventy-one animals were primiparous during the fifth instar and 9 during the sixth. Consequently the number of pre-adult instars is variable, the minimum being four.

Data from the 47 animals which were primiparous during the fifth instar and which lived for twenty instars or more were used in constructing growth curves. Growth in the three dimensions studied is sigmoid. The point of inflection in all curves comes during the fourth instar, the last pre-adult.

The Robertson and the Gompertz equations do not fit the data satisfactorily. This may be due to the time unit employed, which in this case is the instar.

The growth increment is greatest during the fourth instar. The increments increase up to the fourth instar, then decrease gradually until the eleventh instar, after which they remain low and relatively constant.

A significant negative correlation exists between initial body size and initial growth rate, also between duration of growth and final body size. Other characteristics of the growth process investigated appear to vary independently.

The number of young released during the adult instars increases to a maximum at the tenth instar followed by a gradual decrease.

The number of young released during any adult instar is significantly correlated with the growth increment for the instar preceding the one during which the young are released.

Relative growth in the dimensions studied may be expressed satisfactorily by the equation

$$y = bx^{\alpha}.$$

No marked changes occur in the relations between total length and carapace length. Marked changes in the relations between carapace length and height and between total length and height occur at the thirteenth instar.

The standard deviation and coefficient of variation of total length tend to increase somewhat during the early instars, but after the eighth instar both decrease rather steadily.

Relative variability in body size, as measured by the coefficient of

variation of total length, is roughly directly proportional to the logarithm of the growth rate. The existence of this relationship is ascribed largely to the mode of action of environmental factors during growth. It may perhaps also be explicable in terms of Rahn's theory of the physico-chemical origin of variability in growth rate.

Size-frequency analyses of natural populations as methods of studying growth are shown to be inferior to the method using individually reared animals.

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SEASONAL PRODUCTION OF ZOÖPLANKTON OFF WOODS HOLE WITH SPECIAL REFERENCE TO CALANUS FINMARCHICUS[1]

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The purpose of the work described in this paper was the investigation of the distribution of the plankton in the waters around Woods Hole during the summer months and the study of the seasonal changes in the plankton at one locality throughout an entire year. Our primary object specifically was to ascertain whether the copepod, *Calanus finmarchicus*—a form used extensively for laboratory experiments—breeds in this region, and if so during which months and with what success. It was proposed also to include measurements of the physical and chemical factors in the environment, and the collection of nannoplankton and phytoplankton, in order that the sequence of biological events from season to season in this locality might be followed. Cause and effect relationships in the growth of the various plankton forms might thus be unraveled.

Since copepods play a prominent rôle in the economy of the sea, it is important to know what conditions promote their growth and what factors tend to reduce their numbers (Clarke, 1934). To this end a series of laboratory experiments was initiated in which the nutrition of copepods, particularly of *Calanus finmarchicus*, was investigated (Clarke and Gellis, 1935; Fuller and Clarke, 1936; and Fuller, 1937). The nearest point from which copepods of this species could be obtained in sufficient numbers was in the deeper water off Gay Head. The population of *Calanus* at this locality was found to persist throughout the summer, but we had no knowledge either of the subsequent fate of these copepods or of the time and conditions of their appearance here. In our studies of nutrition we had assumed that the *Calanus* found in this area were living in equilibrium with their environment and that the smaller organisms found in the same body of water were adequate both qualitatively and quantitatively for their food supply. If, however, these copepods had been produced in another locality and transported hither by currents, the possibility existed that the conditions at our point of observation were not suitable for the growth or even the maintenance of *Calanus* and that the copepods found here were destined shortly to die off. The proposed field observations were therefore especially desired and when worked out in conjunction with further laboratory experiments should eventually yield information on the factors controlling copepod production which would have a general application.

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[1] Contribution No. 112, Woods Hole Oceanographic Institution.

Let it be understood, however, that the work described in this paper was of the nature of a reconnaissance program. The locality chosen for the observations throughout the year was a point as far offshore as could be reached within a day's sail from Woods Hole, but it probably is not entirely removed from fluctuating land influences. However, evidence will be given below for believing that this station is typical of a considerable area.

Location of Stations and Hydrography

During the summer of 1935 the five stations in the waterways around Woods Hole shown in Fig. 1 were visited about twice a week. Buzzards Bay is a broad, shallow body of water with a temperature a few degrees higher and a salinity slightly lower than Vineyard Sound. A strong current flows in Vineyard Sound, and offshore water seems to be carried into the Sound much more readily than is the case with Buzzards Bay (Haight, 1936). Since access to the open sea from Woods Hole is most direct through the western entrance of Vineyard Sound, a line of stations was run in that direction to study the transition between inshore and offshore conditions and populations.

Station 3, which was selected for the continuation of the observations throughout the year, was visited once a month during the autumn and winter and at least twice a month from April to September. The authors are indebted to Dr. Renn, Mr. Iselin, Mr. Woodcock, and Mr. Butcher for assistance which made these trips possible. A rough sea is almost always encountered at the exposed location of Station 3, and during the extremely cold weather when spray froze on the deck and rigging, work was extremely difficult.

Station 3 is about eight miles from the nearest points of land on the north and east, and twenty-five miles from Block Island on the west. On the south the continental shelf water extends without obstruction. Since at Station H2 (see Fig. 1) the flood tide flows NNE and the ebb tide flows WSW, the main body of water debouching from Vineyard Sound and Buzzards Bay probably passes largely to the west of Station 3. However, the currents in this region are extremely complex and unfortunately the measurements of the U. S. Coast and Geodetic Survey (Haight, 1936) do not extend as far out

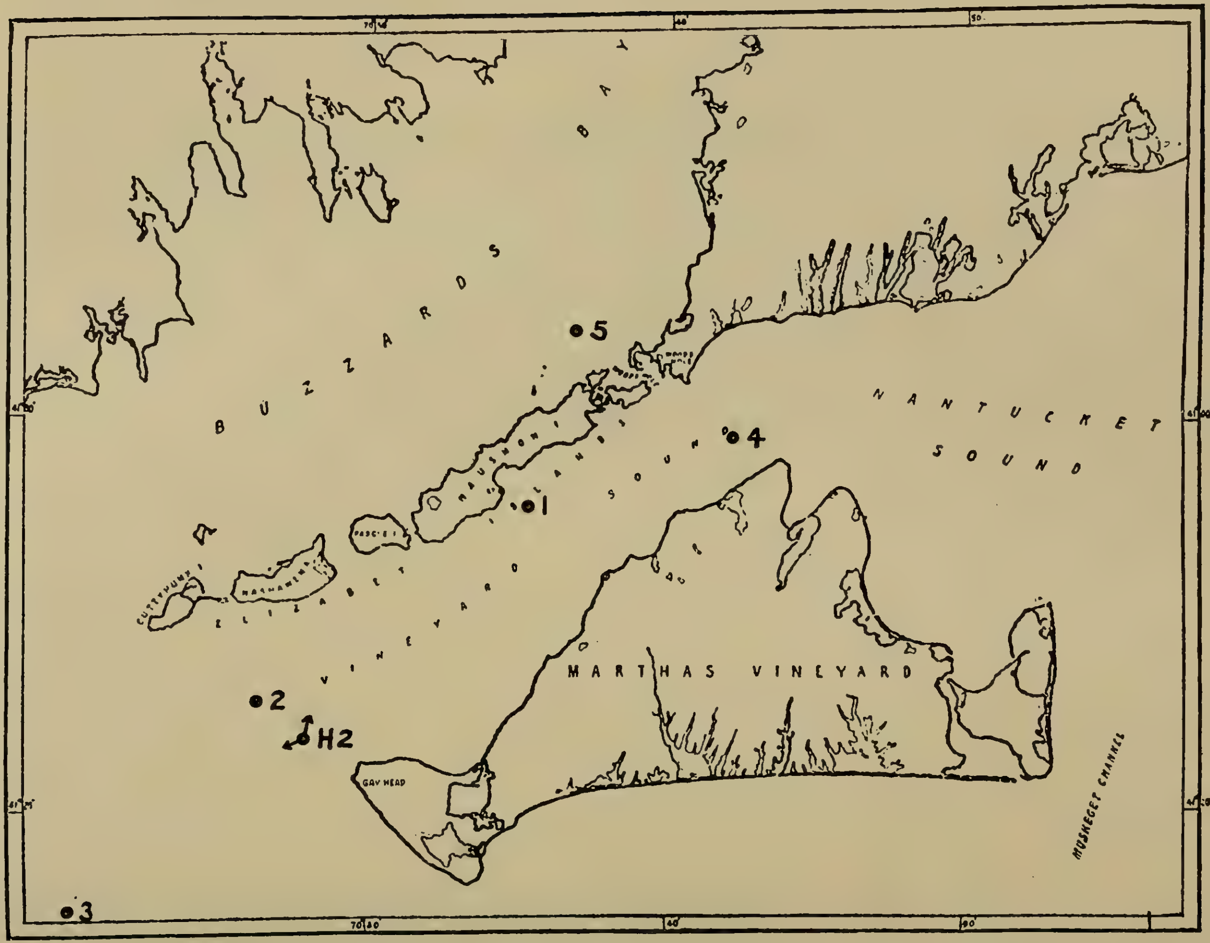
BUZZARDS BAY
NANTUCKET SOUND
VINEYARD SOUND
ELIZABETH ISLANDS
NAUSHON I.
PASQUE I.
NASHAWENA I.
CUTTYHUNK I.
MARTHAS VINEYARD
GAY HEAD
MUSKEGET CHANNEL
5
4
1
2
H2
3
70°50'
40'
30'

FIG. 1. Location of Stations 1–5 at which plankton hauls were made, and of Station H2 occupied by the U. S. Coast and Geodetic Survey. The small island of No Man's Land lies just off the map to the south of Gay Head. Buzzards Bay comes to an end just north of the limit of the map, whereas Nantucket Sound is open to the sea on both sides of Nantucket Island.

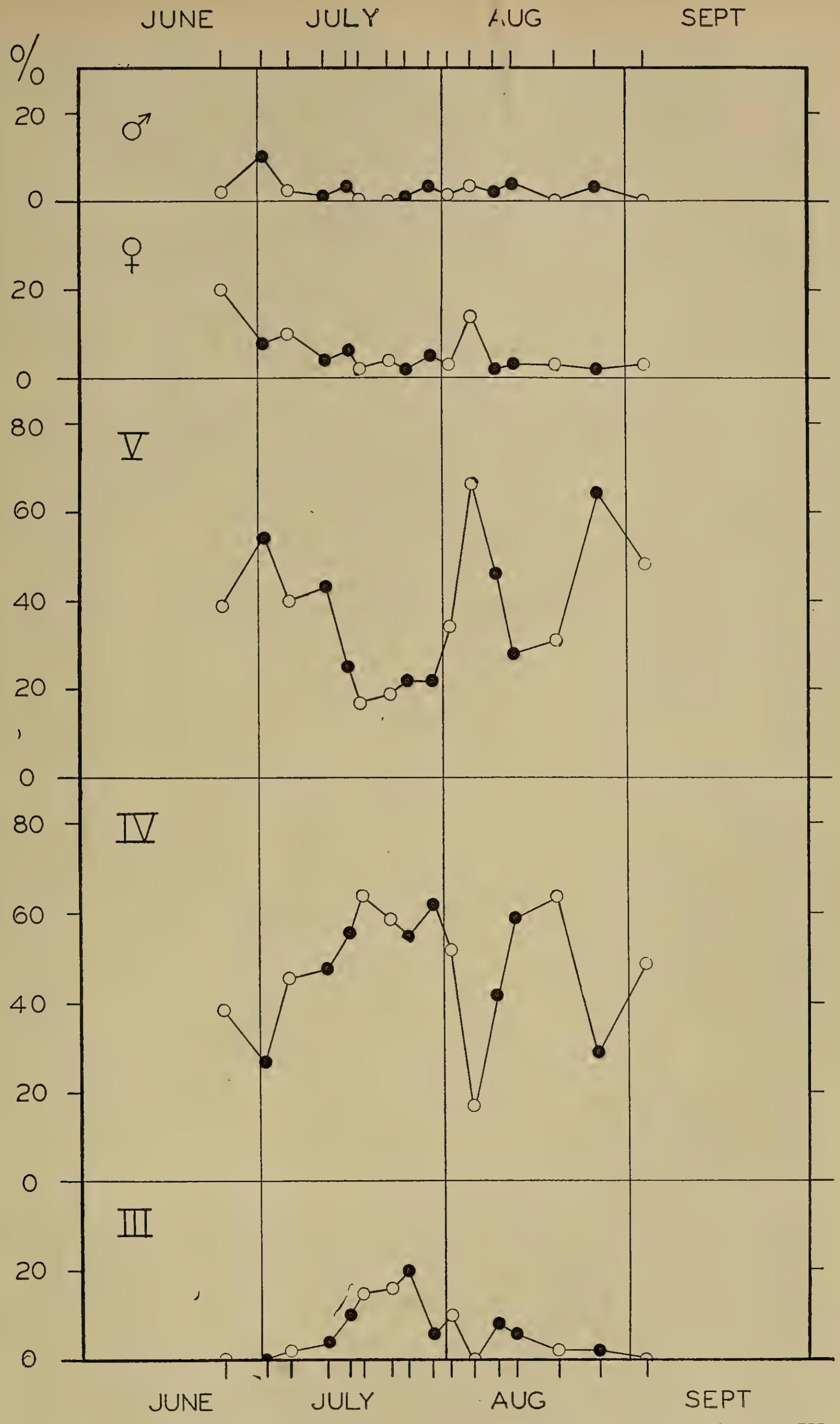

FIG. 2. Percentage distribution of *Calanus finmarchicus* copepodid stages III, IV, and V and adult ♀ and ♂ taken with the scrim net at Station 3 during the summer of 1935. The open circles represent catches made on an incoming tide (E. 1 hour–W. 1 hour) and the solid circles represent catches made on an outgoing tide (W. 1 hour–E. 1 hour).

as our station. Our plankton catches during the summer of 1935 at the stations nearer shore showed a considerable difference depending upon whether they were made on an incoming or an outgoing tide. There is some evidence that the effect of the tide was felt as far out as Station 2, but at Station 3 no consistent difference due to the phase of the tide was observed, as may be seen for the *Calanus* population from Fig. 2. Transparency measurements similarly indicated the presence

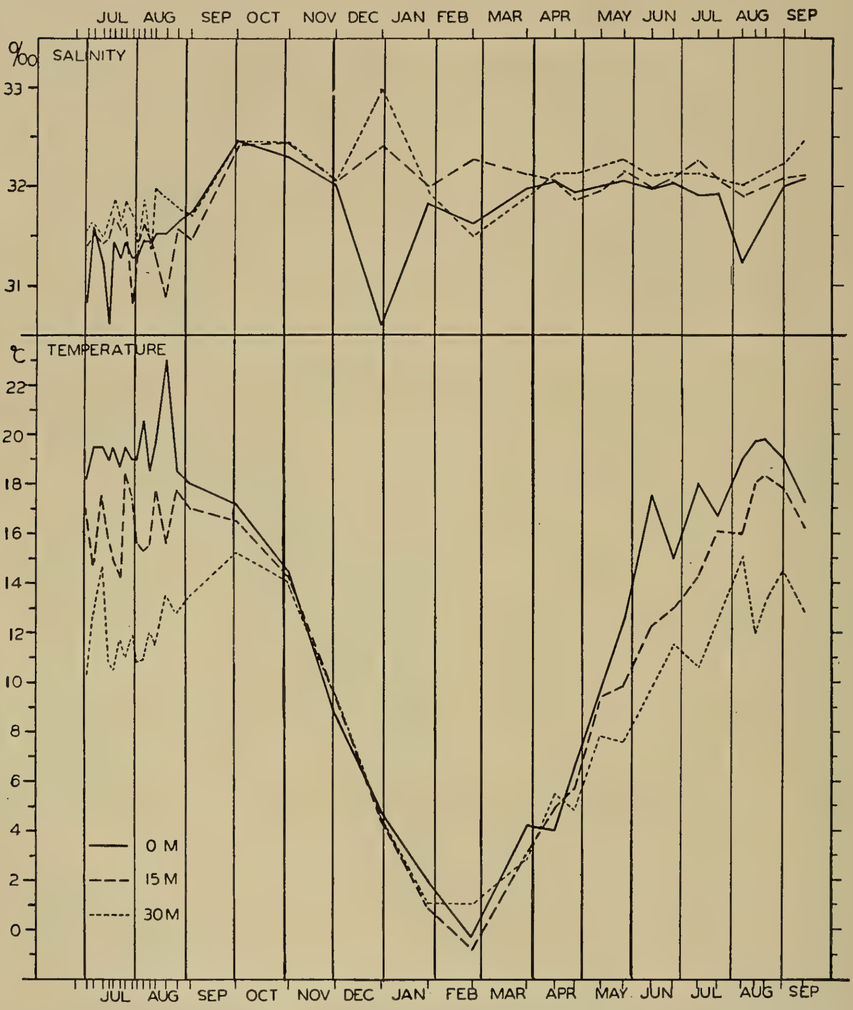

FIG. 3. The salinity and temperature at depths of 0, 15, and 30 meters at Station 3 during 1935–6. The vertical markers along the abscissa indicate the dates on which observations were made.

of typical offshore water at this station (Clarke, 1938). However, for the sake of uniformity, our observations throughout the year were made as far as possible only during the periods when the "east," or flood tide, had flowed for at least one hour, and before the "west" tide had flowed for more than one hour.

These tidal oscillations are superimposed upon the much larger but slower coastal current which flows in a southwesterly direction over the continental shelf all along this coast. Water is moving continuously from Nantucket Shoals on toward New York. It is presumably from the inner edge of this current that the water at our station is derived. Yet there must be a certain admixture of inshore water, since the salinity value at Station 3 (see Fig. 3) was slightly lower at all seasons of the year than that characterizing the water farther offshore[2] and was slightly higher than that found within Buzzards Bay and Vineyard Sound, according to determinations which we carried out during the summer of 1935. The prevailing wind, which is from the southwest, would tend to blow offshore water in toward our station, but gales from this direction or from other points of the compass probably disturb the normal hydrographic situation rather profoundly.

The fluctuations in the salinity at Station 3 from July, 1935 to September, 1936, were for the most part confined between 31.5 and 32.5 ‰ and generally uniform at the depths of 0, 15, and 30 meters (Fig. 3). On several occasions, however, a noticeable freshening of the surface layer was observed, and on December 29, 1935, an unusually great difference in salinity existed between the surface and the bottom. Since a pronounced transparency anomaly occurred on the same day (Clarke, 1938), we may assume that an abnormal interchange of water masses took place on this occasion.

The temperature change at Station 3 during the year was extremely great for a marine environment. The maximum value, observed in August, was over 23° C. higher than the minimum value, in February (*cf.* also Allee, 1919). During the summer months the water was highly stratified, for a difference of 8° or 10° was usually found between the surface and the bottom. This would seem to indicate that no great stirring was produced by wind or tide in these months, but it is conceivable that the temperature difference could be brought about by some persistent differential water movement. In September the surface water began to cool off rapidly and to mix more effectively with the bottom water, thus raising the temperature of the latter. By the first of November the temperature had become uniform from top to bottom. This condition persisted throughout the autumn and winter indicating strong stirring action during this period. In the spring months warming took place more rapidly in the upper layers than in the deeper with the result that a progressively greater difference in temperature was found between the surface and bottom until by June or July a pronounced stratification was re-established.

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[2] At Station "Martha's Vineyard I." See H. B. Bigelow and M. Sears (1935).

METHODS

During the summer of 1935 10-minute horizontal hauls for zoöplankton were made using the scrim closing nets described by Clarke (1933). At Stations 1, 4, and 5 hauls were made at depths of 2 meters below the surface and 2 meters above the bottom; at Stations 2 and 3 an additional haul was made at a depth mid-way between the other two. Water samples were taken and temperatures determined at each of these depths. A fraction of each water sample was turned over to Miss Lois Lillick for qualitative and quantitative analysis of the phytoplankton. Other fractions of the water samples were bottled for determination of salinity, and of phosphate and nitrate content. The authors are indebted to Mr. Alfred Woodcock, Mr. Bostwick Ketchum, and Mr. Homer Smith, for these chemical analyses.

For the work at Station 3 from October, 1935 to September, 1936, the zoöplankton was taken in "oblique" hauls in which the net was lowered to 30 meters (just over the bottom), towed for 1 minute, then raised 1 meter and towed for 1 minute, then raised another meter and so on, until the surface was reached. A double-action hand plankton pump was added to our equipment in order that the eggs, nauplii, and younger copepodid stages of the copepods, which were too small to be retained by the scrim net, might be caught whenever spawning took place. The free end of the hose was attached to the cable about 2 meters above the weight and then lowered to the bottom. The outflow from the pump was piped directly to a 1″ Hersey Water Meter and then discharged into the top of a No. 20 silk phytoplankton net hung vertically in a metal barrel. Ten gallons (about 38 liters) were pumped at each meter from the bottom to the surface.

At 30, 15, and 0 meters bottles were filled from the hose for the chemical analyses and for quantitative phytoplankton counts by Miss Lillick. At 20, 10, and 0 meters the pump was stopped and the contents of the silk net drawn off and bottled separately, thus dividing the catch into three parts representing three strata in depth. On each occasion temperatures were taken and light penetration was measured as is described elsewhere (Clarke, 1938). When time permitted, a second oblique zoöplankton haul and pumping operation were carried out to compare with the first. The net hauls and the pump catches were analyzed by suitable dilution and subsampling (Clarke, 1933). Our thanks are due Mr. David Bonnet for assistance in this task and in the preparation of the tables and diagrams.

GENERAL FEATURES OF THE ZOÖPLANKTON

The number of species of zoöplankton represented in our catches by one or more specimens is enormous (*cf.* Fish, 1925)—too numerous for detailed analysis in an investigation devoted primarily to the study of the production of a few important forms. Even a cursory glance at the material, however, reveals that a considerable difference exists in the plankton at the stations near shore and at those farther out. At Stations 1, 4, and 5 large numbers of larval forms of both bottom-living and pelagic species were encountered mixed in with a varying number of mature individuals of truly planktonic types. The composition of this inshore population changed almost daily in contrast to the more gradual fluctuations which took place offshore.

TABLE I

Total number of Calanus finmarchicus in hundreds calculated on a basis of a 30-minute haul.

Date 1935	Station 1	Station 2	Station 3	Station 4	Station 5
June 24	6	106	90	—	—
July 1	12	65	142	5	37
5	8	90	109	—	0
8	x	91	—	x	15
11	x	108	216	0	4
15	11	152	123	—	—
17	14	74	124	x	x
22	6	20	209	—	x
25	14	288	147	x	x
29	9	529	267	5	3
Aug. 1	84	385	232	3	0
5	5	52	171	6	6
9	11	25	72	—	—
12	3	92	94	x	x
19	8	137	50	x	0
26	0	28	113	0	0
Average	13.6	140.1	143.9	3.1	7.2

— indicates station omitted.
x indicates less than 100.

At Stations 2 and 3 fewer types were found in the plankton and, although quantities of immature specimens appeared at certain seasons, these belonged mainly to the same species as the adult individuals. The difference in the population offshore is well illustrated in Table I, in which the total numbers of *Calanus finmarchicus* at each station are set forth. *Calanus* was consistently scarce at Stations 1, 4, and 5 and, although the average number at Station 3 was only slightly greater than that at Station 2, a population of at least moderate dimensions was always to be found at the offshore station.

TABLE II

Total zoöplankton taken in scrim net at Station 3, 1935–36. Thirty-minute oblique hauls with 75 cm net. Approximate volumes after settling one month.

Date	Haul No.	Vol. cc.	Av.	Date	Haul No.	Vol. cc.	Av.
Sept. 3	181	<10		May 25	204	240	
	182	<10	<10		205	160	200
Oct. 1	185	105		June 11	206	270	
	186	70	88		207	215	246
Nov. 2 (Sta. 2)	187	215		June 25	208	105	
	188	160	188		209	140	123
Dec. 1	189	140	140	July 10	210	70	
Dec. 29	191	105 S			211	90	80
	192	160 S	134	July 22	212	45	
Jan. 27	193	105 S			213	40	43
	194	185 S	145	July 30	214	240	240
Feb. 23	195	700 S	700	Aug. 6	215	210	210
Mar. 27	197	10	10	Aug. 14	216	70	
Apr. 13	198	40			217	70	70
	199	65	53	Aug. 20	218	210	
Apr. 25	200	115			219	160	185
	201	115	115	Aug. 31	221	70	
May 11	202	65			222	40	55
	203	90	78	Sept. 13	223	<10	
					224	10	10

Note: Hauls mostly copepods except those marked "S" which were mostly sagittæ. The net used catches effectively animals as large as, or larger than, copepodid Stage IV of *Calanus finmarchicus.* Many smaller forms are not retained by the net.

The seasonal variation in the total population may be traced from the approximate volumes of the zoöplankton taken at Station 3 throughout the year (Table II). Plankton was scarce during September and October, but increased in November and December. The mid-winter hauls were characterized by large quantities of sagittæ. After this plankton again became scarce, but during the spring months volumes increased reaching a maximum early in June

The plankton remained abundant through the summer with few exceptions until in September a sudden reduction was encountered.

At all the stations Crustacea—usually copepods—formed the bulk of the catch with the exception of a few occasions on which large numbers of medusæ or, at the offshore stations, of sagittæ were encountered. The most common copepods, in addition to *Calanus finmarchicus*, which will be treated in a separate section, were the following (*cf.* also Fish, 1925):

Centropages typicus	July to Dec.	Numerous in August
Centropages hamatus	May to July, and in Sept. and December	Numerous in June and July
Pseudocalanus minutus	Jan. to Oct.	Numerous Mar. to Aug.
Paracalanus parvus	July to Sept. and in Feb.	Numerous in Aug.
Acartia tonsa	April to Dec.	
Oithona similis	April to Sept. and probably throughout the year	
Labidocera æstiva	Oct. to Dec.	

Many of these copepods appear to breed in this region because immature specimens of most of the list were found on one occasion or another. The copepodid stages of *Centropages* (species not determined) were particularly numerous during the summer months, and the locality is a veritable nursery for *Pseudocalanus* as judged by the large numbers of eggs, nauplii, and copepodites taken in the pump catches, particularly in the spring.

Sagitta elegans was represented by at least a few specimens in almost every haul at Station 3 and the large catches of sagittæ reported on several occasions consisted entirely of this species. *Sagitta enflata* occurred in small numbers in October and November, 1935, and in August, 1936. *Sagitta serratodentata* was taken from September to December, 1935, and in August, 1936, and was abundant on only one occasion. It was noticed that the size of *Sagitta elegans* varied greatly from month to month and that the smallest individuals appeared in January, May, July, and September. Since these months agree almost exactly with the four periods of the year during which Russell (1932–33) believes the main breeding of *Sagitta elegans* to take place at Plymouth, we may conclude that the seasonal production of this species is approximately the same on both sides of the Atlantic.

THE PRODUCTION OF CALANUS FINMARCHICUS

Comparison of Net and Pump Catches

The numerical analysis of our zoöplankton hauls was limited almost entirely to *Calanus finmarchicus*. The numbers of this species taken in the scrim net ran into the tens of thousands. If we assume 100 per cent straining efficiency, the scrim net filtered 564,000 liters of water during the standard 30-minute haul. In the standard pump operation, on the other hand, the total volume of water delivered to the phytoplankton net in pumping from 30 meters to the surface amounted to 1134 liters. One copepod in the pump catch would theoretically correspond to about 500 copepods in the net haul provided that the two methods are equally effective in catching all stages of *Calanus*. This was not expected to be the case, but since a few of the older copepodites and adults were usually to be found in the pump catches, these older stages were counted as well as the younger stages in order that the sampling efficiency of the pump and the net might be compared.

The total number of each stage of *Calanus* in the pump catches throughout the year is shown by the solid areas in Fig. 4. All the nauplius stages have been lumped together because the duration of each of these stages is short compared to the intervals between our observations. Very few of the earlier nauplius stages were taken. Certain eggs, which may have been those of *Calanus*, were found, but the number of these was so trifling as not to be worth plotting. The scarcity of eggs and early naupliï suggests that the actual spawning ground may be farther offshore than our station.

The numbers of *Calanus* taken in the net hauls is indicated by the superimposed single line (Fig. 4), but using a different scale. The magnitude of the scale which has been used for the net hauls was determined by finding the ratio between the two sets of data for copepodid Stage IV. This stage was chosen for the comparison of the numbers taken with the pump and with the net because it seems to have been caught effectively by both methods. Since the number of *Calanus* in this stage was high both on July 30 and August 6, the average number taken by the net on these two days was divided by the average number taken with the pump. The resulting quotient was very nearly 100. Accordingly all the totals for the net hauls were divided by 100 before plotting. The scrim zoöplankton net should theoretically remove animals from 500 times as much water as the pump. Since the volume of water passing through the pump was accurately measured, we reach the conclusion that the straining efficiency of the scrim net is 20 per cent.

The two curves for Stage IV which have been brought together at one point in the manner described are seen to correspond very roughly for the rest of the year. For copepodid Stage V and for the adults, however, the curves for the pump catches consistently lie below those for the net hauls. This indicates that the latter method is relatively more effective than the former for these larger individuals either

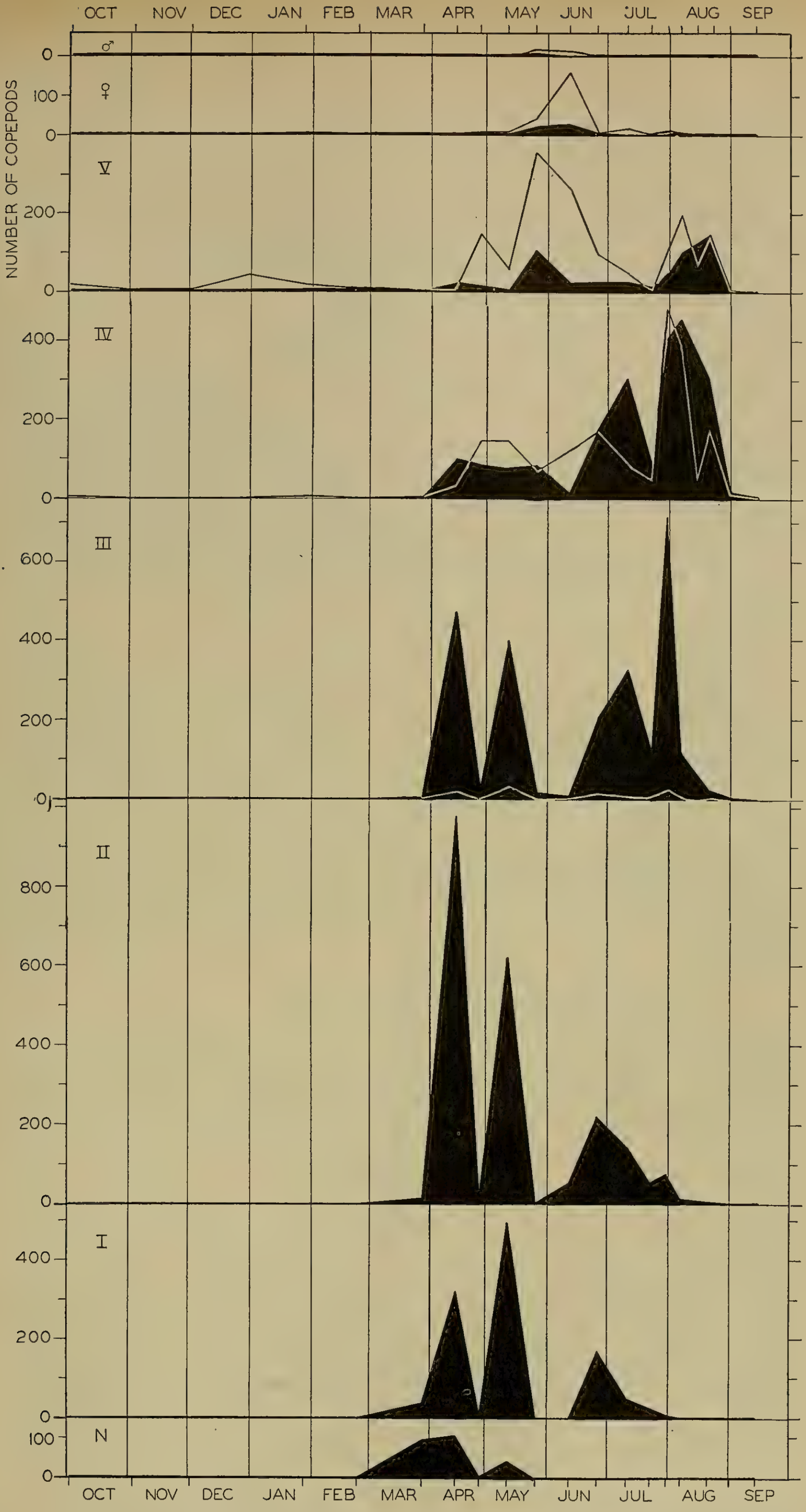

FIG. 4. The solid black area indicates the total numbers of *Calanus* naupliï (N), copepodid stages I–V, and adult ♀ and ♂ taken with the pump per standard haul (see text). The superimposed line shows the numbers of *Calanus* taken with the scrim net per standard haul, but for these the scale must be multiplied by 100.

because fewer slip through the mesh of the net or because more escape the mouth of the hose. The opposite situation is seen in the case of copepodid Stage III in which it appears that a disproportionate number of this group fails to be caught by the net.

Seasonal Changes in Abundance

Nauplii occurred in significant numbers on only three occasions, namely March 27, April 13, and May 11. Copepodid Stages I and II have peaks of abundance on April 13, May 11, and June 25. Stage III was numerous also on April 13 and May 11 and a third period of abundance extended from the end of June to the first part of August. The numbers of individuals in Stage IV were moderate in April and May, increased rapidly in June, and reached a maximum early in August. The net caught representatives of Stage V in every haul throughout the year, but with the exception of December 29, numbers were extremely low from September until the end of April. This stage was particularly abundant at the end of May and again in August, but never equalled the maximum of Stage IV. Adult specimens of *Calanus* were most numerous in May and June, but at all times were relatively scarce, particularly the males. Taking the species as a whole, we may conclude that the population is of very small dimensions during the autumn and winter. By the first of April reproduction has begun and from then on through the summer *Calanus* is abundant. At some time after the end of August the species suddenly becomes depleted and the population is not restored again until the following spring.

Succession of Generations

To determine the number of generations[3] which succeed one another during the year recourse is best made to the percentage distribution of the various age stages within each of the hauls from season to season because this procedure removes the confusing effect of fluctuations in the size of the total catch and allows the progression from stage to stage to stand out in relief. The percentage diagram for the pump catches (Fig. 5) shows that on the first of October the total catch

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[3] The term "generation" is used here instead of "brood," which has been employed by others in this connection, because the former word expresses correctly the relationship of the two groups of copepods which appeared during the course of the year. The term "brood" should be limited to its strict sense in order to avoid confusion. Nicholls (1933) regards it possible for each female copepod to produce more than one brood of ova. Accordingly there may be circumstances in which the young of the second generation exist contemporaneously with a late second brood of the first generation. The necessity for using these terms with their exact meanings thus seems obvious.

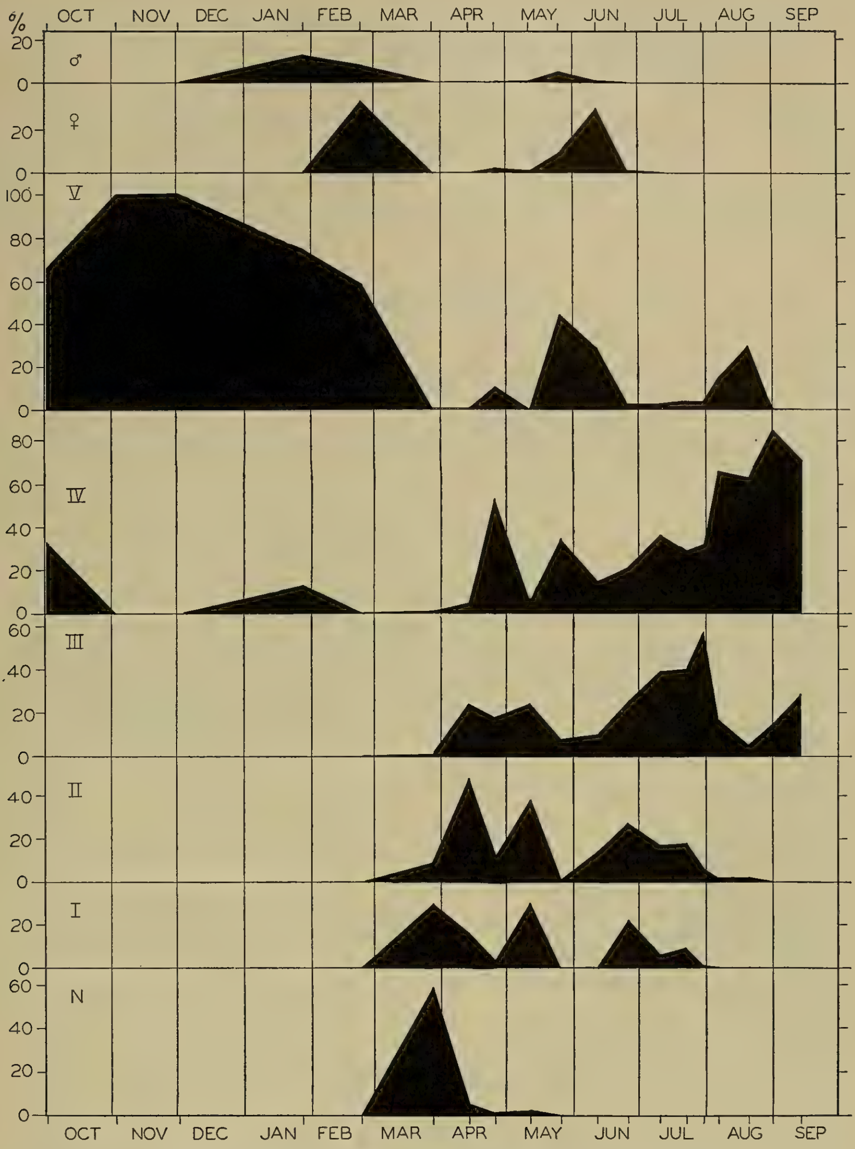

Fig. 5. Percentage distribution of *Calanus* nauplii, copepodid stages I–V and adult ♀ and ♂ taken with the pump at Station 3 during 1935–6.

consisted of 66 per cent Stage V and 34 per cent Stage IV. During November and December practically 100 per cent of the species occurred as Stage V. But in January some of these individuals matured into adult males and in February an even larger proportion of females appeared. A month later the males and females had largely disappeared and Stage V was reduced to 1 per cent, while nauplii comprised 60 per cent of the catch and copepodid Stage I amounted to 30 per cent. During April other copepodid stages appeared in succession, but these gave rise to only a very small percentage of adult females and practically no males. In May a second series of peaks is to be found progressing up through the copepodid stages and culminating in a peak for the adult males on May 25 and a prominent peak for the females on June 11.

The relationship between these two series of peaks in April and May is obscure. Although we know that in other areas two or more generations may follow one another in rapid succession during the spring and summer, the time interval between our two series appears to be too short for them to represent successive generations. In laboratory experiments Nicholls (1933) found that 27 days was the minimum time possible from the shedding of the ova to the appearance of the adults at the temperature at which he worked (apparently 11–14° C.). The observations of Ruud (1929), Lebour (1916) and Fish (1936) indicate that a longer time than this is required for the entire development of *Calanus*. Marshall, Nicholls, and Orr (1934) state that "the eggs appear after two to four weeks, spawning may last for several weeks, and the adults then die out." Taking fourteen days as the minimum time for the maturation of the eggs and 27 days as the minimum for development, it appears that 41 days is the shortest interval which can exist between the spawning of one generation and the spawning of the next—or between the two generations at any corresponding stage of development. At certain stages in our April and May series the peaks are less than 30 days apart and the temperature of the water at the time was only about 6–8° C. It seems very doubtful, therefore, that the copepodites appearing in May were produced by the individuals which were found as copepodites in April. It is possible that the May group is a late second brood from the same parents as the April group. Adult specimens of *Calanus* were present in the water throughout April although their numbers were low. Another possible explanation of the situation is that at the end of April an unusually extensive movement of water along the coast swept away the population which had reached Stage IV on April 25 and brought in an entirely distinct population of copepods whose development was two or three weeks behind that of the first group. The reduction in the abundance of the first group when it reaches Stage V and its practical disappearance thereafter support this idea (*cf.* Fig. 4).

Another group of Stage I copepodites appeared late in June and continued into July, but in lesser numbers. In the succeeding weeks the older copepodid stages became numerous one after another until by August 20 a prominent peak had been built up in Stage V. This summer generation was undoubtedly produced by the individuals which we observed as mature adults in May and June, although the

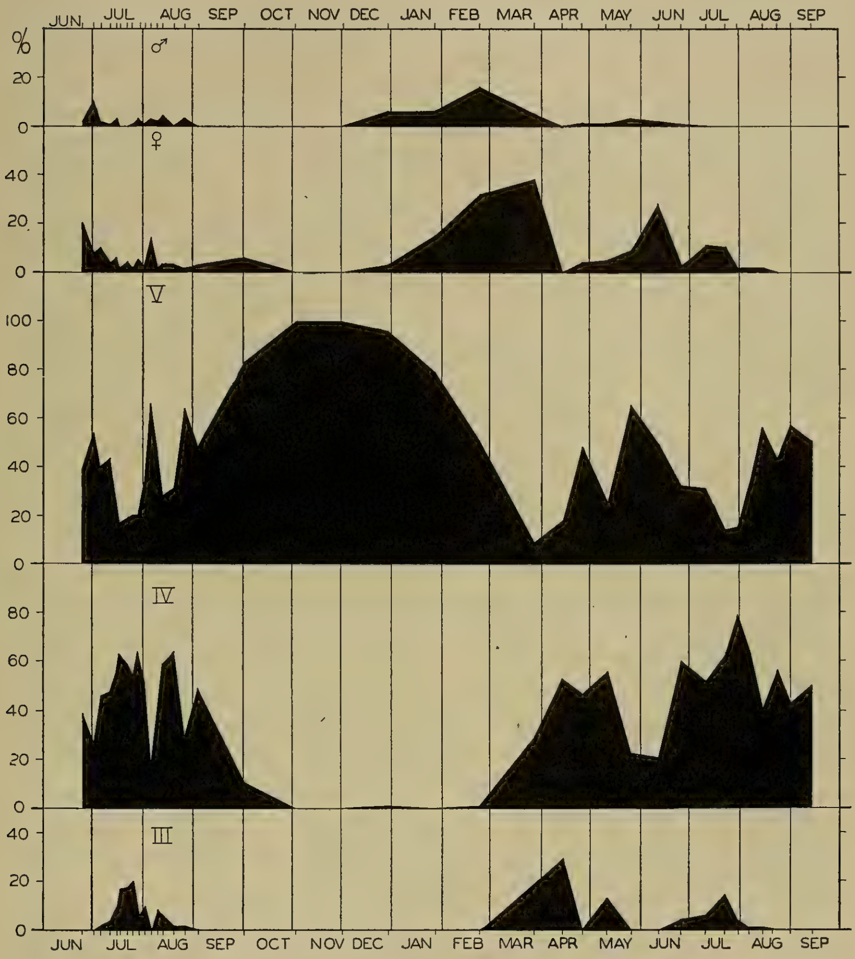

FIG. 6. Percentage distribution of *Calanus* copepodid stages III, IV, and V and adult ♀ and ♂ taken with the scim net at Station 3 during 1935–6.

eggs and naupliï which should have appeared in June were not found. Evidently the spawning took place during the two-week's interval between our visits to the station in June, or else at a point farther offshore. There is some indication in the diagram that this summer generation consisted of more than one brood, but the irregularities were so great that no definite conclusion can be reached. It seems clear, however, that Stage IV moulted only slowly into Stage V during August and that these individuals did not mature into adults immediately, but formed the population destined to carry over the autumn and winter as Stage V.

The catches with the scrim zoöplankton net have similarly been calculated on a percentage basis to serve as a check on the conclusions reached with the pump catches (Fig. 6). The numbers of the older stages of *Calanus* taken with the pump were low and the possibility existed that the larger individuals escaped the 1-inch opening of the hose, whereas loss from this cause would be much less in the case of the scrim net. Since the net hauls were begun four months earlier than the work with the pump, we have a record for two summers and the intervening winter. At the end of June, 1935, relatively large numbers of adult males and females were found. The spawning of these produced the summer generation which appeared as Stage III copepodites in July. This generation formed the winter stock which did not mature until January, February, or March. Individuals in Stage III appearing in the net hauls in April (with a subsidiary group in May) constituted the spring generation of 1936. This generation matured and spawned in June and we find the summer generation as Stage III in July, thus nicely confirming the net haul results of the previous year.[4] As before, these individuals grew slowly during the summer, and when work was discontinued in September, the population was about equally divided between Stage IV and Stage V.

From both the pump catches [5] and the net hauls we conclude that at Station 3 a short-lived generation of *Calanus* occurs during the spring and is followed by a long-lived generation which has its origin in the early summer and lasts through the following winter. One or more subsidiary groups were found during the spring and summer which may represent second broods from the same adults, or immigrants in a different stage of development, or possibly additional generations of very short duration.

These two main breeding periods correspond closely with those observed by Fish (1936) in the Gulf of Maine for the "western stock" of *Calanus*. Fish believes that this stock forms the principal source of supply for the Gulf and he reports that these animals breed chiefly in March–April and in June–July, but possibly also in September. The developmental period of two and one-half months which Fish calculated for his *Calanus* agrees well with the approximate interval between our spring and summer generations. Marshall, Nicholls, and Orr (1934) found that in the Clyde Sea three generations of *Calanus* occurred during the course of a year. The spawning periods for these

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[4] During the summer of 1937, however, incidental observations showed that the *Calanus* population was extremely small in this region and consisted mostly of Stage V. Evidently annual variations may be considerable.

[5] The fact that the pump catches obtained through a 1-inch hose gave the same general picture of the production of *Calanus* as the net hauls suggests that a device with a relatively small opening is adequate for plankton studies and could be more widely used (*cf.* Hardy, 1936). Such a device would have the important advantage that the volume of water strained could be accurately measured.

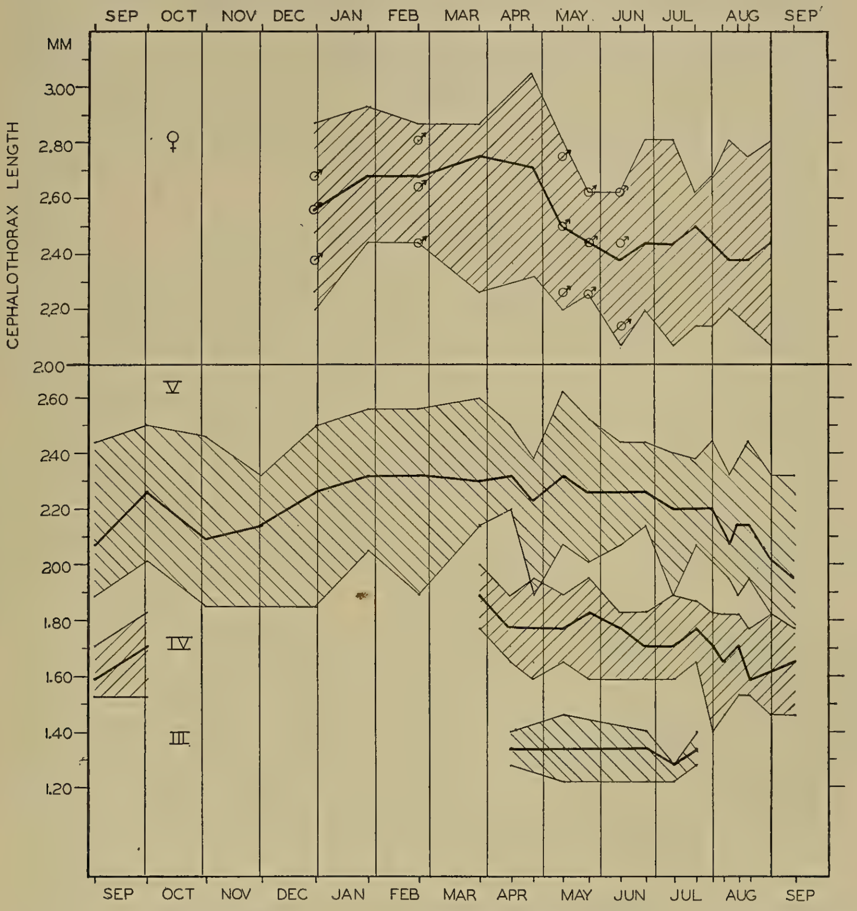

FIG. 7. Cephalothorax length of *Calanus* copepodid stages III, IV, and V and adult ♀ at Station 3 during 1935–6. The shaded area indicates the range and the heavy line the mean of 25 measurements. Since males were sufficiently numerous to measure on only five occasions, the limits of the range and the mean are indicated by the ♂ symbols placed on the upper diagram. It will be noted that the scales of the upper and lower diagrams overlap to a certain extent.

generations took place in March, May, and June. Our observations differ only in that the batches of nauplii appearing off Woods Hole from March to May seemed to belong to the same generation. Bogorov (1934) reports that three generations occur in the Plymouth area. He believes spawning to take place in February–March, May, and intermittently from June to August. Other investigators (Paulsen, 1906; Bigelow, 1926; Farran, 1927; Russell, 1928; Ruud, 1929; and Sømme, 1934) have found a similar succession of two or more generations in their respective areas, but since thorough reviews have been made by Fish (1936) and by Nicholls (1933), their results will not be discussed here.[6]

Variation in Size

The cephalothorax length of 25 specimens of copepodid Stage V was measured for each haul throughout the year and for Stages III and IV and for the adults on those occasions when these groups were sufficiently numerous (Fig. 7). The range of the measurements for each group in each haul was greatest for the adults and progressively less for the younger stages. The average length of Stage V did not fluctuate widely but was found to be somewhat greater from January to May than during the rest of the year. Stage IV similarly diminishes in size after the spring months have been passed. The adult males and females averaged about 2.65 mm. in length from January through April, but in May a sudden drop occurred to about 2.45 mm. and this smaller size persisted through the summer.

The abrupt reduction in the size of the adults in May coincides with the appearance in the hauls of the mature males and females of the new spring generation (Figs. 4 and 5). These individuals were spawned at the end of April or early in May when the temperature ranged between 5° and 9° C. We have already seen that the generation which was produced in June at temperatures of 11° to 15° C. apparently did not reach maturity until the following January or February. Consequently, the summer generation resulted in larger adults than the spring generation although the latter group was spawned at a temperature about 6° C. lower. This relationship with temperature is just the reverse [7] of that reported by Marshall, Nicholls, and Orr (1934) and by Bogorov (1934). These investigators found that the individuals of the spring generation were the largest of the year and Bogorov reported that the females of this generation were 1.2 times larger than those of the autumn-winter generation.

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[6] Results of a similar nature have been reported by Wimpenny (1936) in a publication which appeared since this paper went to press.

[7] The adult females which were taken in April and which were of large size may possibly have been spawned in March and, if so, present a case of correlation of large size and low temperature during the spawning period.

Comparison with Phytoplankton and Nannoplankton

The phytoplankton obtained in the water samples taken at 2, 15, and 30 meters on each occasion has been analyzed by Lillick (1937). She reports that during the summer of 1935 the phytoplankton was dominated first by *Chætoceros* and associated forms and later by *Rhizosolenia.* On October 1 the diatoms reached a maximum.[8] During the rest of the autumn and early winter the phytoplankton was extremely poor. Late in January numbers increased somewhat but by the end of February the phytoplankton was scarce again. On no occasion was a typical spring diatom maximum encountered but Miss Lillick cites evidence for believing that a great flowering of diatoms took place early in February at a time between two visits to the station. During the late spring and summer of 1936 the phytoplankton remained at a low ebb, with *Guinardia* appearing as the dominant form, until August 9 when for a period of four days a flowering of *Rhizosolenia* and *Guinardia* occurred.

Diatoms were the most prominent element of the flora at all times in this region, but dinoflagellates appeared in smaller numbers throughout the year especially in summer and fall. In addition Miss Lillick lays stress upon a group of flagellated and pigmented forms which appeared in all the samples in very large numbers. Although this group was made up entirely of exceedingly small forms, she states that their frequency was so great as to make them significant in the phytoplankton population and she believes that "they must doubtless play an important part in the general food cycle of the region."

Special water samples for the study of the bacterial population were taken by Dr. C. E. Renn at 5-meter intervals from the surface to the bottom on each trip to Station 3 from October 1, 1935 to May 26, 1936. The numbers of cells per cc. as determined by plate counts ranged from 30 to 350 during the first three months but from February to May no counts greater than 33 were obtained (Renn, 1937). Although the actual numbers of bacteria in the sea may have been a thousand times greater than this, we have shown elsewhere that populations of these dimensions cannot be a significant source of food for *Calanus* (Fuller and Clarke, 1936). Besides the bacteria, and the pigmented forms mentioned above, to which Miss Lillick confined her attention, other nannoplankton types undoubtedly existed—possibly in significant quantities. The presence of great numbers of these, during the summer months at least, has been demonstrated by Lackey (1936). In most routine investigations many of the smaller and more delicate forms are probably overlooked because the usual preservatives destroy them or render them unrecognizable.

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[8] Our observations on the zoöplankton were not made at sufficiently short intervals to determine conclusively whether or not this flowering coincided exactly with a marked reduction in the number of grazing animals (*cf.* Harvey, Cooper, Lebour, and Russell, 1935). In 1935 a sharp drop in the number of *Calanus* occurred on Nov. 2; in 1936 a similar diminution of numbers took place on August 31.

Since Marshall, Nicholls, and Orr (1934) found that in Loch Striven the periods of diatom increases coincided with the three main spawning periods of *Calanus*, it is interesting to examine our data for similar correlations. For this purpose observations should have been taken at much shorter intervals than was possible in the present investigation. Conclusions are also made difficult in our case because of our lack of information in regard to the state of the fauna and flora in contiguous areas and the effect which horizontal movements of water masses would produce. It is clear, however, that whatever diatom flowering may have occurred in the early spring months between two visits to the station, this could not have served as a food supply for our *Calanus* since naupliï did not appear until March 27. On this date and throughout the ensuing four months diatoms were not abundant. Similarly no production of *Calanus* was observed at the times of the secondary diatom maximum in August or October. The phytoplankton which did exist during the breeding periods of *Calanus* may be studied from Table III. Diatoms were relatively numerous on April 13 and May 11, occasions when naupliï and early copepodites were abundant,

TABLE III

Phytoplankton at Station 3, March to June, 1936. The number of cells per liter at 2, 15, and 30 meters have been averaged.

	Mar. 27	Apr. 13*	Apr. 25	May 11*	May 25	June 11	June 25
Diatoms	33	9,450	600	4,700	1,467	2,300	700
Dinoflagellates	67	100	1,300	0	67	0	3,767
"Flagellates"	300	5,750	1,433	950	2,633	7,533	6,767
Other forms	0	350	0	0	67	100	167
Total	400	15,650	3,333	5,650	4,234	9,933	11,401
Calanus							
Naupliï	Abundant	Abundant		Abundant			
Copepodid I–III		Abundant		Abundant			Abundant

* Average of two depths only.

but they were scarce on March 27 when the first nauplii of the season appeared and on June 25 when the early copepodites of the second generation came into prominence. On this latter date dinoflagellates were especially numerous. The forms designated as "flagellates" were abundant throughout this period.

Although our data are not sufficient to allow us to conclude which are the essential food organisms for *Calanus* nor in what quantity they are required, nevertheless we have obtained certain facts which limit the possibilities. We have seen that the spawning periods of *Calanus* occurred at times which did not coincide with diatom maxima. We know from Miss Lillick's analysis that throughout the year the diatoms and dinoflagellates appeared suddenly at intervals of a week or two, flourished for a few days only, and then disappeared again. The "flagellates," on the other hand, although small in size, were found in relatively large numbers on all occasions. If *Calanus* feeds chiefly on diatoms, we must conclude either that the small number of cells always present as a minimum is sufficient for their nutrition, or that the food obtained by the copepods at times of local flowerings can be converted into reserve tissue (e.g. oil) which will tide them over until the next period of diatom abundance. The number of diatoms present between flowerings seems too low to fulfill the nutritive requirements of *Calanus* (Fuller and Clarke, 1936; Fuller, 1937) but further investigations may show this not to be the case. As regards the second possibility, we know that *Calanus*, in the later copepodid and adult stages at least, can live for a week or two without food, but is unable to moult successfully under these conditions (*loc. cit.*). The copepods might thus be able to survive without moulting from flowering to flowering—and this may be the actual situation during the autumn and winter—but rapid growth such as took place from April to July would probably be impossible unless adequate nutriment was continuously available. A third possibility which our present observations suggest is that the nannoplankton is important as food for copepods. The "flagellates" which were found at Station 3 at all times in large numbers would represent only a small amount of substance because of their minute size, but if, as seems likely, these pigmented forms are only a small fraction of the total nannoplankton, the latter may turn out to be significant as a food source.

SUMMARY

1. The seasonal production of zoöplankton, particularly of *Calanus finmarchicus*, was investigated by means of scrim nets and a plankton pump at five stations in the vicinity of Woods Hole during the summer

of 1935 and at one offshore station throughout the ensuing year. Collections of phytoplankton and of nannoplankton and measurements of temperature, salinity, phosphates, nitrates, and illumination were carried out at the same time.

2. The zoöplankton consisted largely of copepods, but medusæ, sagittæ, and, at the stations near shore, larvæ of both benthonic and planktonic forms occurred irregularly. *Sagitta elegans* exhibited four main breeding periods during the year.

3. The numerical analysis of the *Calanus* population revealed the presence of nauplii in significant numbers in March, April, and May, and of early copepodites in April–May and June–July. Stage V was found at all seasons. Adult specimens were relatively scarce at all times, but most abundant in May and June. The species as a whole was reduced to small numbers during the autumn and winter.

4. The pump catches and the net hauls agree in indicating that a short-lived generation of *Calanus* occurs during the spring and that this is followed by a long-lived generation. The latter has its origin in the early summer, passes through the autumn and winter as Stage V, and matures early the following spring to give rise to the next short-lived generation.

5. The measurement of the cephalothorax length of 25 specimens in each stage older than copepodid Stage II showed that the average lengths of the various groups did not fluctuate widely during the year except in the case of the adults which exhibited a sudden drop in size in May.

6. The spawning periods of *Calanus* did not occur at times of diatom maxima, and therefore the two phenomena are not directly related in the present case. In regard to the nourishment of this copepod we must conclude (*a*) that the small number of diatoms always present as a minimum is sufficient, or (*b*) that the animals build up sufficient reserve on occasions of small local flowerings of diatoms, which occur at intervals of a week or two, to tide them over the intervening periods, or (*c*) that "flagellates," which were found continually in large numbers, and other types of nannoplankton are important as a food source.

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SEASONAL STUDIES OF THE PHYTOPLANKTON OFF WOODS HOLE, MASSACHUSETTS[1]

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During the summer of 1935 certain investigations of the plankton in the waters near Woods Hole, Massachusetts, were undertaken by workers at the Oceanographic Institution. The zoöplankton studies have been described in the preceding paper by Clarke and Zinn (1937); the observations on phytoplankton form the subject of the present paper. At the outset five stations were established for regular observations, four in Vineyard Sound, and one in Buzzard's Bay. For the phytoplankton work water-bottle samples were taken at each station twice weekly, and at three depths, surface, mid-depth, and bottom. Along with these, determinations of the temperature, salinity, nitrate and phosphate content of the water were made.

During the first two months the water at all five stations was found to be so homogeneous in nature with regard to the phytoplankton, that it seemed unprofitable to continue observations at all stations. Therefore all subsequent collections were made at Station 3 alone, which is located at the Whistle Buoy near the western entrance to Vineyard Sound, Latitude 41° 17′ 35″, Longitude 71° 0′ 0″ (see Clarke and Zinn, 1937, for map). This station was chosen because conditions there, more nearly than at any of the others, simulate those of open waters, as will be seen later. From September, 1935, through the winter months samples were taken once a month; from April through July, fortnightly; during August, weekly. The final phytoplankton data were collected on August 20, 1936, giving a range of fourteen months for the entire survey. Samples were taken at the 2-meter, 15-meter, and 30-meter levels, the bottom being at 32 meters.

In this paper only such forms have been included as fall naturally within the plant classes, the chlorophyll-bearing or food-producing groups, which include the *Cyanophyceæ*, *Diatomaceæ*, *Dinoflagellatæ*, *Silicoflagellatæ*, *Coccolithineæ*, and certain of the pigmented flagellated groups. All of the true protozoan classes have been omitted.

In making the quantitative determinations of the phytoplankton for the majority of the samples Gran's centrifuging method was used, modified somewhat by having the organisms concentrated over a membrane filter, and then centrifuged. Since by this method others have found that there may be a 10 per cent loss of organisms, an original sample of 1,100 cc. was used instead of 1,000 cc. to allow for this error. For the winter and spring samples the precipitation method of Nielsen and von Brand (1934) was used chiefly because the amount of sample available was not sufficient to permit the use of the other method.

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[1] Papers from the Department of Botany of the University of Michigan, No. 629; Contribution No. 156 of the Woods Hole Oceanographic Institution.

Most of the collections were made by Mr. Donald Zinn and the crew of the Oceanographic Institution boat *Asterias*. Chemical determinations were made by Dr. Homer P. Smith and Mr. Bostwick Ketchum. The writer is indebted to Dr. H. B. Bigelow and Dr. F. K. Sparrow, Jr., for certain guidance at the Woods Hole Oceanographic Institution, and to Dr. William R. Taylor and Professor H. H. Bartlett under whose direction the work was completed at the University of Michigan.

HYDROGRAPHIC OBSERVATIONS

Certain physical features of the water, currents, tides, temperature, and salinity, play an undoubtedly important part in the distribution of plankton. The observations made on these factors during the present investigation have been presented by Dr. Clarke (Clarke, 1937; Clarke and Zinn, 1937). Although the control of phytoplankton species distribution, at least in part, must be attributed to the influence of temperature and salinity, no obvious correlations could be made between the variations in these factors and in the seasonal distribution of phytoplankton.

Nitrate determinations ranged from 4 milligrams of N. as NO_3 per cubic meter of sea water at 15 meters on October 1, to 62 milligrams at 2 meters on December 29 (see Figs. 1, 2, and 3). Through the summer months of the first year, the nitrate content of the upper strata of water was low, exhibiting slight fluctuations. Somewhat higher values were obtained in the lower strata. During the winter nitrates were gradually built up throughout the entire water mass to their highest concentration in early January. It is noteworthy that there is a decided lag in this accumulation of nitrates behind that of phosphates, a feature which is consistent with Cooper's theory (1933) based on observations in the English Channel, that the phosphates are returned directly to the water upon the decomposition of the plankton; whereas the nitrates are regenerated only after a series of intermediate steps. Terminating the winter accumulation, there was a sudden dropping off in the amount of nitrates in late January and early February, followed by a more gradual decrease throughout March and April. The summer was marked by small amounts of nitrates with

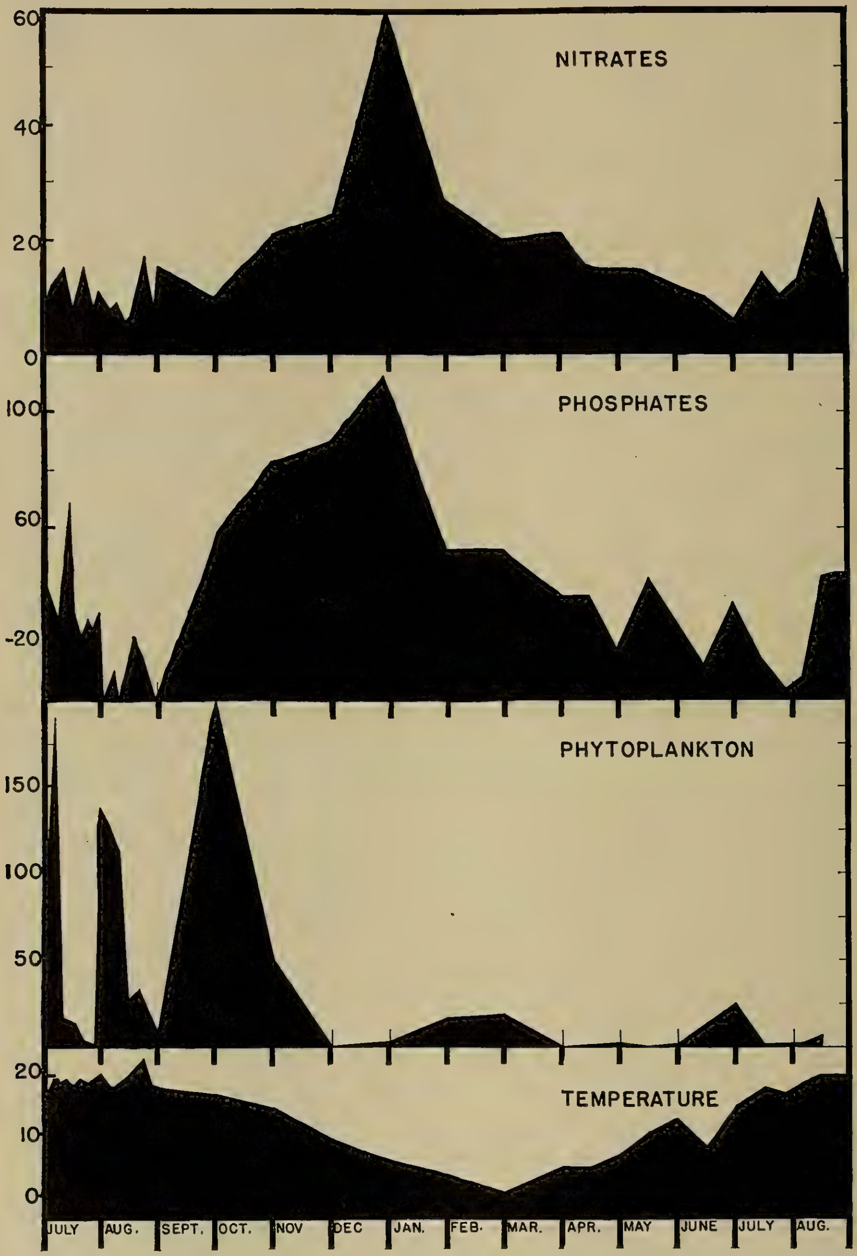

FIG. 1. Distribution of nitrates, expressed as milligrams of N as NO_3 per cubic meter of water; phosphates, as mg. of PO_4 per cubic meter; phytoplankton, in thousand cells per liter; and temperature, in degrees centigrade, at 2 meters depth, Station 3, from July, 1935, to Aug., 1936.

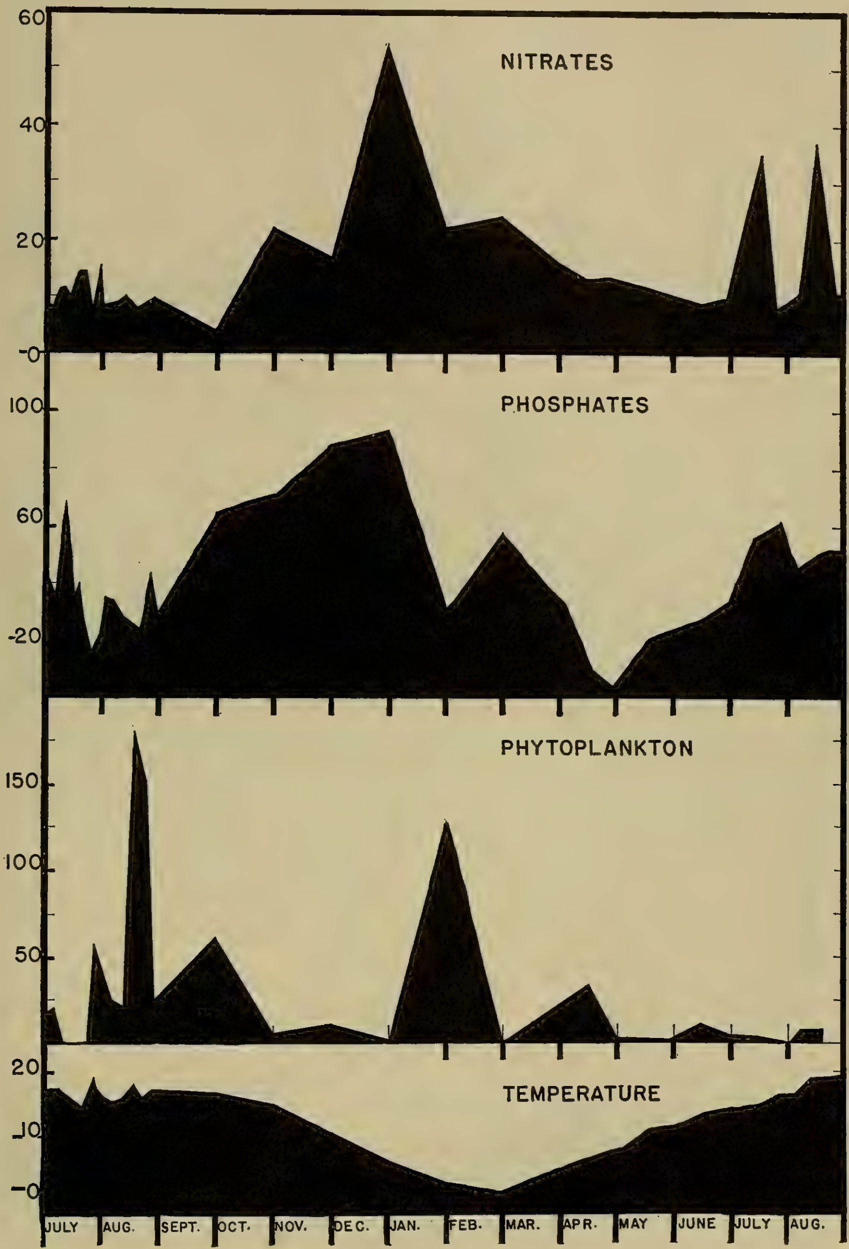

FIG. 2. Distribution of nitrates, expressed as milligrams of N as NO_3 per cubic meter of water; phosphates, as mg. of PO_4 per cubic meter; phytoplankton, in thousand cells per liter; and temperature, in degrees centigrade, at 15 meters, Station 3, from July, 1935, to Aug., 1936.

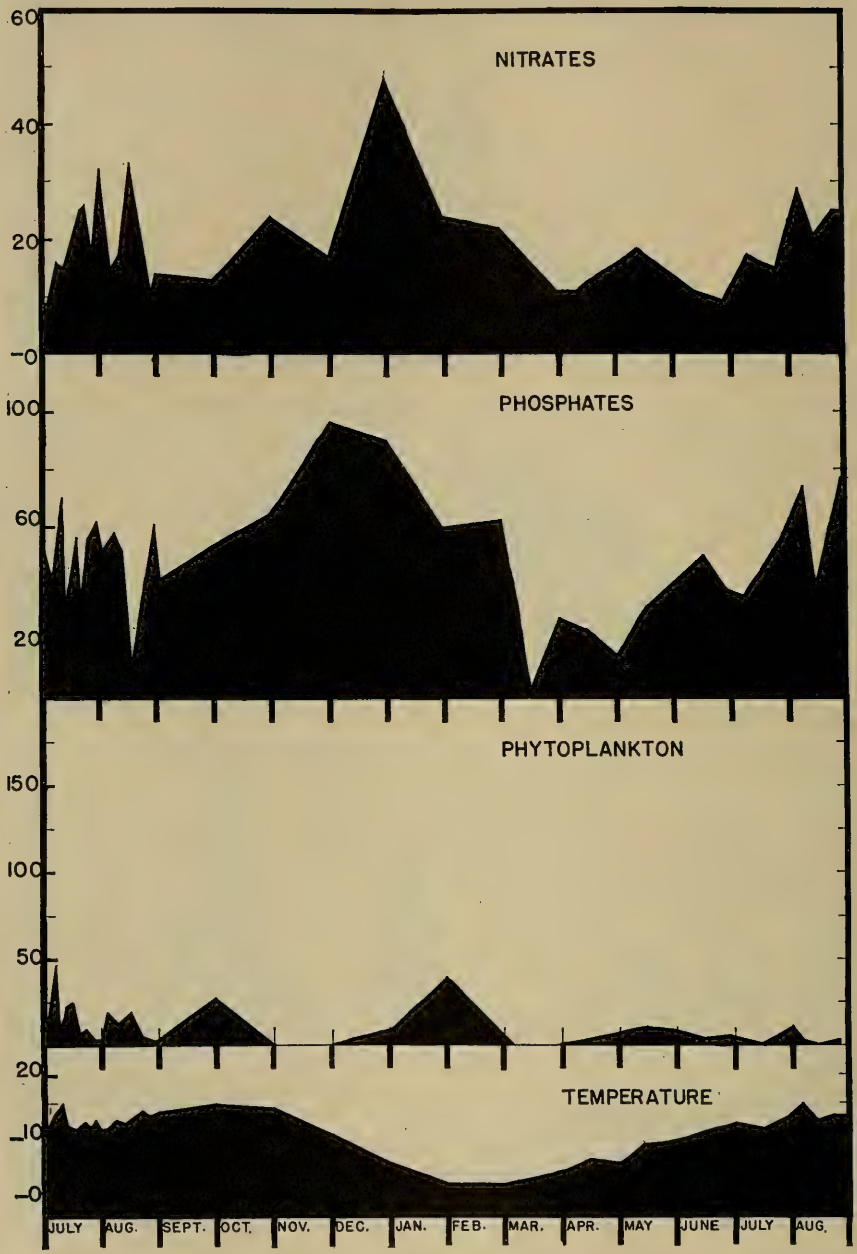

FIG. 3. Distribution of nitrates, expressed as milligrams of N as NO_3 per cubic meter of water; phosphates, as mg. of PO_4 per cubic meter; phytoplankton, in thousand cells per liter; and temperature, in degrees centigrade, at 30 meters, Station 3, from July, 1935, to Aug., 1936.

only slight fluctuations. In August there was an accumulation to form a second lesser peak, which again lagged behind the phosphates, and which was followed immediately by a decrease in concentration. Variations with depth were never extremely pronounced. However, there was usually some degree of stratification. From May through August, the nitrates tended to become concentrated at the bottom. By September they were more or less uniformly distributed, and remained so until December, when the surface waters became very rich in nitrates, the bottom waters much less so. This general relationship continued through April.

The phosphate distribution differed somewhat from that of nitrates. The total amounts found in the water were always much higher, though the total range was greater, from none in the surface waters through most of August and September, to 112 mg. PO_4 per cubic meter at the surface in December. The seasonal phosphate cycle was as follows: low through July and August of the first summer; increasing through September and October; reaching a maximum during the winter months of November, December and January; decreasing from the end of January through February and March, and dropping to the lowest point for the year in April, remaining low through May and June; in July and August gradually increasing, with another drop at the end of August. Stratification of phosphates was frequently much more pronounced than that of nitrates. From June through August the phosphates were found to be more concentrated at the bottom. In October the waters became more nearly uniform throughout, and remained so until spring, when the amounts at the surface increased over those at lower layers, this relationship remaining until June. The possible correlation of phytoplankton production with nitrates and phosphates will be discussed later.

PHYTOPLANKTON

(See Tables I and II)

The larger part of the phytoplankton in our collections was made up of diatoms, dinoflagellates, and flagellates. During certain seasons of the year members of the Silicoflagellatæ and Coccolithineæ became rather numerous, and stray members of the Cyanophyceæ appeared in the collections on occasion. More than 100 different species appeared during the year, divided among the several groups as follows:

Diatomaceæ	57
Dinoflagellatæ	31
Silicoflagellatæ	3
Coccolithineæ	2
Cyanophyceæ	2
Flagellatæ	?

TABLE I

Seasonal distribution of the more important phytoplankton species at 15 meters, Station 3, July, 1935, through August, 1936.

Species	Cells per Liter											
	July 5	July 15	July 25	Aug. 1	Aug. 12	Aug. 26	Sept. 3	Oct. 1	Nov. 2	Dec. 1	Dec. 29	Jan. 27
Diatomaceæ	....	....	....	....	....	....	....	....	....	....	....	....
Asterionella Bleakleyi	....	....	....	....	598	228	....	....	....	....	....	....
Chætoceros spp.	....	....	180	....	184	500	....	....	4,000	....	....	....
Ch. borealis	....	....	....	....	184	46	....	2,300	....	....	....	....
Ch. constrictus	....	....	1	....	138	1,496	....	500	....	....	....	....
Ch. decipiens	270	....	....	....	....	....	....	900	....	....	....	....
Ch. didymus	....	....	....	....	....	....	....	2,200	....	....	....	....
Corethron hystrix	....	....	....	138	36,708	2,048	....	....	....	....	....	....
Guinardia flaccida	....	....	....	322	1,196	....	....	100	600	200	....	....
Leptocylindrus danicus	....	....	....	....	4,874	....	....	2,300	....	700	....	....
L. minimus	....	....	....	....	42,964	....	....	300	....	....	....	....
Melosira sulcata	225	....	....	1,485	....	228	....	660	600	300	....	....
Nitzschia Closterium	90	....	46	....	3,108	228	....	....	....	....	....	....
N. seriata	135	....	....	....	14,306	368	....	17,600	3,600	5,800	....	....
Rhizosolenia alata	....	....	....	....	....	....	....	1,300	....	....	....	....
R. calcar-avis	....	....	....	138	....	....	....	....	100	....	....	....
R. fragillissima	....	....	....	....	....	....	....	800	....	100	....	....
R. hebatata semispina	270	....	46	....	....	....	....	300	200	....	....	330
Sceletonema costatum	....	....	....	506	46,782	1,035	....	30,000	....	....	....	....
Thalassionema nitzschioides	7,965	92	....	230	2,392	46	....	1,200	....	700	900	500
Thalassiosira Nordenskiöldii	225	....	....	1,196	276	405	....	800	....	300	300	3,800
Dinoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Gonyaulax tamarensis	90	....	....	....	322	....	1,300	....	....	....	....	....
Peridinium spp.	....	....	....	....	92	1,588	....	100	....	....	....	....
P. simplex	....	....	....	....	3,184	774	....	....	....	....	....	....
Prorocentrum minimum	6,480	92	598	3,780	2,832	2,502	....	....	....	....	....	....
P. Scutellum	....	....	....	368	966	319	....	....	....	....	....	2,160
Silicoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Distephanus speculum	1,755	....	....	184	....	....	....	100	....	....	....	....
Coccolithineæ	....	....	....	....	....	....	....	....	....	....	....	....
Syracosphæria	....	....	....	....	....	....	100	....	....	....	....	....
Flagellatæ	1,215	....	....	2,852	9,338	8,681	900	500	300	1,100	500	1,992
Total at 2 meters	189,000	16,000	35,000	136,000	30,000	19,400	9,300	214,000	53,000	1,000	4,900	16,000
Total at 15 meters	20,000	400	1,240	11,000	181,000	26,000	3,500	63,000	6,800	11,000	1,700	127,000
Total at 30 meters	45,000	19,000	9,800	2,900	21,000	2,070	....	28,000	500	200	7,300	39,000

TABLE I—*Continued*

Species	Cells per Liter											
	Feb. 23	Mar. 27	Apr. 13	Apr. 25	May 11	May 25	June 11	June 25	July 10	July 22	Aug. 6	Aug. 14
Diatomaceæ	....	....	....	....	....	....	....	....	....	....	....	....
Asterionella Bleakleyi	....	....	....	....	....	....	....	....	....	....	....	....
Chætoceros spp.	....	....	....	....	....	....	....	....	....	....	....	....
Ch. borealis	....	....	....	....	....	....	....	....	....	....	....	....
Ch. constrictus	....	....	....	....	....	....	....	....	....	....	....	....
Ch. decipiens	....	....	....	....	....	....	....	....	....	....	....	....
Ch. didymus	....	....	....	....	....	....	....	....	....	....	....	....
Corethron hystrix	....	....	....	....	....	....	....	....	....	....	....	500
Guinardia flaccida	....	....	18,500	....	....	....	....	....	....	300	300	1,200
Leptocylindrus danicus	....	....	....	....	....	....	....	....	....	....	....	....
L. minimus	....	....	....	....	....	....	....	....	....	....	....	....
Melosira sulcata	....	....	....	....	....	....	....	....	600	....	300	700
Nitzschia Closterium	....	....	....	....	....	....	....	....	....	....	....	....
N. seriata	....	....	....	....	....	....	....	....	....	....	....	....
Rhizosolenia alata	....	....	....	....	....	....	....	....	....	....	....	....
R. calcar-avis	....	....	....	....	....	....	....	....	....	....	700	700
R. fragillissima	....	....	....	....	....	....	....	....	....	....	....	....
R. hebatata semispina	200	....	....	....	....	....	....	....	....	....	....	....
Sceletonema costatum	....	....	....	....	....	....	....	....	....	....	....	....
Thalassionema nitzschioides	....	....	100	....	....	....	200	....	....	....	....	1,000
Thalassiosira Nordenskiöldii	....	....	....	....	....	....	....	300	500	....	....	500
Dinoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Gonyaulax tamarensis	....	....	....	....	....	....	....	....	....	....	....	....
Peridinium spp.	....	....	....	....	....	....	....	....	....	....	....	....
P. simplex	....	....	200	....	....	....	....	....	....	....	....	....
Prorocentrum minimum	....	....	....	....	....	....	....	200	200	....	800	100
P. Scutellum	....	....	....	....	....	....	....	....	100	....	200	200
Silicoflagellatæ	....	....	....	....	....	....	....	....	....	....	....	....
Distephanus speculum	....	....	....	....	....	....	....	....	....	....	....	....
Coccolithineæ	....	....	....	....	....	....	....	....	....	....	....	....
Syracosphæria	....	....	600	....	....	....	....	100	....	....	1,000	....
Flagellatæ	....	....	9,500	1,800	....	1,000	10,200	1,200	1,200	....	3,200	1,400
Total at 2 meters	19,000	500	2,300	2,800	200	2,500	15,600	26,000	2,500	2,600	2,800	7,150
Total at 15 meters	200	700	29,000	1,900	....	1,000	11,000	2,900	2,000	300	6,800	7,000
Total at 30 meters	....	....	....	5,700	11,000	9,200	3,300	5,900	1,300	12,000	300	800

TABLE II

Complete list of the species found at Station 3 during the survey of 1935–1936.

DIATOMACEÆ
- *Achnanthes tæniata*
- *Asterionella Bleakleyi*
  - *japonica*
- *Bacteriastrum hyalinum*
- *Cerataulina Bergonii*
- *Chætoceros* spp.
  - *affinis*
  - *borealis*
  - *borealis* var. *concavicornis*
  - *compressus*
  - *constrictus*
  - *curvisetus*
  - *debilis*
  - *decipiens*
  - *didymus*
  - *gracilis*
  - *laciniosus*
  - *teres*
- *Cocconeis placentula*
- *Corethron hystrix*
- *Coscinodiscus centralis*
  - *excentricus*
  - *lineatus*
- *Grammatophora marina*
- *Guinardia flaccida*
- *Hemiaulus Hauckii*
- *Leptocylindrus danicus*
  - *minimus*
- *Lichmophora abbreviata*
- *Melosira moniliformis*
  - *sulcata*
- *Navicula* spp.
- *Nitzschia Closterium*
  - *longissima*
  - *seriata*
- *Pleurosigma Normanii*
- *Rhabdonema adriaticum*
- *Rhizosolenia alata*
  - *calcar-avis*
  - *fragillisima*
  - *hebatata* var. *semispina*
  - *imbricata* var. *Shrubsolei*
  - *setigera*
  - *styliformis*
- *Sceletonema costatum*
- *Striatella unipunctata*
- *Surirrella Gemma* ?
- *Synedra Gallionii* ?
- *Thalassionema nitzschioides*
- *Thalassiosira decipiens*
  - *gravida*
  - *Nordenskiöldii*
- *Thalassiothrix Frauenfeldii*
  - *longissima*

DINOFLAGELLATÆ
- *Ceratium Fusus*
  - *lineatum*
  - *longipes*
  - *longipes* var. *oceanicum*
  - *macroceros*
  - *Tripos*
- *Dinophysis acuminata*
  - *norvegica*
  - *Ovum*
- *Exuviælla baltica*
- *Glenodinium trochoideum*
- *Gonyaulax tamarensis*
- *Mesoporos asymmetricus* [2]
- *Minusculus bipes*
- *Noctiluca miliaris*
- *Peridinium* spp.
  - *breve*
  - *Cerasus*
  - *conicoides*
  - *conicum*
  - *denticulatum*
  - *depressum*
  - *gracile*
  - *Granii*
  - *simplex*
- *Phalocroma irregulare* ?
- *Prorocentrum micans*
  - *minimum*
  - *Scutellum*

SILICOFLAGELLATÆ
- *Dictyocha Fibula*
- *Distephanus speculum*
- *Ebria tripartita*

COCCOLITHINEÆ
- *Rhabdosphæra tubulosa*
- *Syracosphæra* spp.

FLAGELLATÆ

CYANOPHYCEÆ
- *Glœocapsa*
- *Oscillatoria*

Both oceanic and neritic forms occurred with several important species belonging to either class. Neritic forms included both pelagic and bottom types; and occasional tychopelagic forms appeared.

The summer flora (see Table I) of 1935 was broadly typical of the area for that season. In July various species of *Chætoceros* were important. This is the normal summer flora for regions lying to the north of Woods Hole, and to be expected here. This genus was accompanied by *Corethron hystrix*, *Guinardia flaccida*, *Leptocylindrus danicus* and *Nitzschia seriata*, all temperate or boreal forms. In August these forms were replaced by species of *Rhizosolenia* (*R. calcar-avis*, *R. alata*, and *R. setigera*) and *Thalassionema nitzschioides*, a temperate flora. The summer maximum was reached during August, and consisted of *Rhizosolenia* and *Guinardia*. These are such large forms that the numbers recorded give a false impression of the actual state of the water. Plankton nets drawn through the waters during one of these maxima became clogged within a few minutes with *Rhizosolenia*. This summer maximum is very typical of the general area, and is similar to that found by Fish (1925). The entire flora

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[2] Since the name *Porella* given by Schiller (1928) to a genus of the family Prorocentraceæ, Dinoflagellatæ, is identical with that of the well known liverwort genus *Porella* [Dill.] L., order Jungermanniales, and since this latter group antedates Schiller's genus by a great many years, it seems necessary to change the name of the dinoflagellate group. For this genus the name *Mesoporos* is offered here in substitution for *Porella* Schiller. Since Schiller's generic name was not established by a Latin diagnosis, one is given here for the nomenclatorial validation of the genus, in accordance with the International Rules.

Mesoporos gen. nov.

Porella Schiller non *Porella* [Dill.] L.

Cellula ovalis plusminusve lateraliter applanata. Flagella per fenestram edentatam poriferem exserta. Hemitestae poro singulo mediano intus projecto praeditae, si lateraliter viso invaginationem conicam obtusam apice perforatam formanti. Chromatiphora dua vel tria flava vel brunneo-flava ad hemitestas applanata, absque pyrenoideis. Species 5, a cl. Jos. Schiller sub nomen Porellam descriptae, Arch. f. Protistenkunde 61: 54. 1928.

Mesoporos bisimpressus (Schiller) comb. nov.

Exuviælla bisimpressa Schiller, Arch. f. Protistenk. 38: 257. 1918. *Porella bisimpressa* ibid., **61**: 54. 1928.

Mesoporos asymmetricus (Schiller) comb. nov.

Porella perforata (Gran) Schiller, *Arch. f. Protistenk.*, **61**: 55. 1928. *Porella asymmetrica* Schiller, Dinoflagellata, Bd. 10, 3 Abt., S. 29, in Rabenhorst's Kryptogamen-flora. 1931.

Mesoporos globulus (Schiller) comb. nov.

Porella globula Schiller, *Arch. f. Protistenk.*, **61**: 56. 1928.

Mesoporos adriaticus (Schiller) comb. nov.

Porella adriatica Schiller, *Arch. f. Protistenk.*, **61**: 56. 1928.

Mesoporos perforatus (Gran) comb. nov.

Exuviælla perforata Gran, 1915, Bull. plankt. for 1912. Cons. Perm. int. p. l'explor. de la Mer, Copenhagen.

for this season was much more characteristic than that found in the following summer.

The winter flora, following the fall maximum, was extremely poor (see Table I). Collections made on October 1 showed a relatively large number of diatom species, occurring in considerable abundance. These collections were very similar to Fish's (1925) typical winter flora and indicate that the season of 1935 resembled those of 1922 and 1923 at that time. In October 1935 the species which Fish had found to be dominant were again dominant, namely, *Rhizosolenia alata*, *Sceletonema costatum*, and *Leptocylindrus danicus*. Certain of the forms which he mentions as occurring in abundance were found in our collections to be important here in October and at no other time throughout the winter (*Chætoceros decipiens* and *Rhizosolenia setigera*). This similarity is surprising in the face of the total lack of similarity throughout the rest of the winter.

For the rest of the winter months our collection showed that the plankton as a whole was extremely poor. The four important species, and at times nearly the only species found, were *Thalassionema nitzschioides*, *Rhizosolenia hebatata* var. *semispina*, *Thalassiosira Nordenskiöldii*, and *Nitzschia seriata*. All are boreal forms, the first two oceanic, and the last two neritic. Nearly all of the supplementary species were likewise boreal. Along with these forms there was a relatively high percentage of flagellated forms, a group which appeared throughout the year and which will be discussed later. These results are in the main very different from those of Fish (1925), who found a definite winter "maximum" consisting of a greater variety of abundant species than the summer.

It is extremely unfortunate that the great difficulty in making these collections necessitated the spacing of the trips rather far apart. During much of the year, because of the relative stability of the flora, this should have made little difference. Nevertheless it has resulted in our failing to observe any especial spring flowering of diatoms which may have occurred and which is so characteristic of the region north of Cape Cod. It is well known from the work of Bigelow, Gran and Braarud, and others that at some time during the early spring, and for a period of short duration, there is a flowering of diatoms dominated by *Thalassiosira Nordenskiöldii* in the Gulf of Maine, Bay of Fundy, and Massachusetts Bay, which results in phytoplankton numbers far in excess of those of any other season of the year. Fish seems not to have found a similar flowering, though he mentions *T. Nordenskiöldii* as occurring throughout January, February and March. However, the conditions with which he dealt at the Bureau of Fisheries' dock were far different from those at our more off-shore station. He does, however, record the occurrence of a winter maximum beginning in November or December and lasting into March, for Vineyard Sound and Long Island Sound. Whether or not a flowering of either type occurred at our station during the spring of 1936 must remain problematic; certainly no such conditions appeared in our collections. It is entirely possible that in the interval between any two of our spring collections such a flowering could have burst forth and disappeared again without showing up at all in our collections. The weight of evidence which we have at hand seems to indicate that if such a flowering occurred during the spring of 1936, it could not have happened in all probability later than early February. Collections taken at the end of January show that the phytoplankton had increased decidedly over the winter months, yet was not abundant enough to be considered as a "flowering." Further, there was a decided increase in the number of cells of *T. Nordenskiöldii.* Other species which began to appear or to become prominent were *Rhizosolenia fragillissima*, *Nitzschia seriata*, *Thalassiosira decipiens*, *Thalassionema nitzschioides*, and certain of the peridinians, all boreal forms of the neritic or oceanic type.

By the end of January the nitrates and phosphates in the water had decreased markedly from their winter maximum concentration in December. Similar sudden depletions of these salts have been found by others in the English Channel, Loch Striven, and elsewhere (Harvey, 1928; Atkins, 1927; Marshall and Orr, 1929) to be coincident with the outburst of spring phytoplankton. It seems logical to infer that such an explanation may account for the sudden decrease in these salts at our station. The collections of late February and the following months show a marked falling off of the phytoplankton, and of the critical species, a condition which would normally follow the spring maximum. All of these arguments combine to indicate that any spring flowering of diatoms which may have occurred at our station must have done so between the end of December and the earlier part of February.

Following February the plankton became exceedingly scarce, and entered the summer period, which for 1936 was the poorest for the entire year. A few species of *Chætoceros*, which usually make up a large portion of the summer flora in this latitude, and which were prominent the year before, appeared early in the season but died out as the year progressed. The important species throughout the season was *Guinardia flaccida*, a temperate neritic form. The flagellates also became prominent for a time in June, and then decreased. Temperate forms became more common; whereas boreal forms tended to die out. Certain of the smaller dinoflagellates appeared during the summer, though never in great numbers. The summer maximum, which is of very short duration, did not show up in our collections. However, we do have other information which gives the time and nature of this summer flowering. Collections made in another connection at the Oceanographic Institution showed that during a period of three or four days, from August 9 to August 11 or August 12 the waters in Vineyard Sound were teeming with a diatom flora which excluded nearly everything else, coloring the water a deep olive green. An examination showed this flowering to be made up almost entirely of the two large diatom species, *Rhizosolenia calcar-avis*, and *Guinardia flaccida*, both of which were prominent in the summer maximum of the year before. This growth appeared very suddenly and disappeared just as rapidly. As can be seen from our collections of August 6 and August 14, both species were present in the waters, but in very small amounts. Table I shows the general occurrence and abundance of the more important species found throughout the fourteen months at a depth of 15 meters.

Diatoms were obviously the most important element of the flora throughout the year. Dinoflagellates appeared throughout the year, more frequently in summer and fall, but their numbers were never sufficient to outweigh the diatoms. A group of forms which were exceedingly small, flagellated and pigmented, which therefore must be considered in a treatment of phytoplankton, kept appearing in all of our collections throughout the year, and in very large numbers. The formaldehyde used to preserve the collections apparently so distorted these forms that identification was impossible. There is no question that many widely separated organisms appeared in this class, which have been lumped together indiscriminately here under the term "Flagellatæ." At times certain of the forms were obviously swarm spores of certain of the diatoms and peridinians. It is quite possible that others may have been spores of other algal groups. Some were evidently independent flagellate forms. Much difficult work will be necessary before this general group can be clearly understood. No mention of such a prominent group as we have found seems to have gotten into the literature of this general region, probably because much of the previous work has been done with a no. 20 plankton net, which would allow these small types to slip through the meshes. Poor though our general grouping of the forms may be, it seems desirable to include them in this account because they doubtless play an important part in the general food cycle of the region.

In Table II is given a complete list of the phytoplankton found to occur at Station 3, during the survey.

Broadly speaking, then, our results correspond well with those of Fish, whose work is the only one of a similar nature conducted in a nearby region. Making allowances for the great differences between the two localities, and for the limits placed upon our collections, the seasonal cycle of the two surveys corresponds remarkably well. Comparison of the species given in Table II with those listed by Fish shows that a certain number of littoral or bottom forms appeared in his collections and not in ours. A number of these species appeared in our collections made during the first summer at the more littoral stations. Other features characteristic of more offshore waters are shown by our station. Along with stratification of the water column is seen a certain degree of stratification of phytoplankton. One of the outstanding characteristics of Fish's station is the homogeneity of the water from surface to bottom. The seasonal cycle at Station 3, if we consider that we have sufficient data from which to judge, more nearly resembles that of offshore waters, with its spring and fall maxima, and summer and winter lulls, than it does the neritic waters. Conditions here must be considered to be somewhat intermediate between the other two types.

For a long time oceanographers have held to the theory that phytoplankton production is tied up with and dependent upon the amounts of nitrates and phosphates found in the water in any given latitude. In a broad sense, although this does not seem to be the final factor which controls the cycle, there must be, certainly, some close correlation between the two. Some such broad relationship can be seen in the results of our survey. An examination of Figs. 1, 2, and 3 will show that the nitrates and phosphates were relatively plentiful during July and early August of 1935, and that they dropped suddenly during August. They gradually accumulated during the fall and rose to their highest point during the winter months. During January there was a sudden drop to a summer low which continued until July, when the salts began to accumulate again to a lower peak in August. Another decrease occurred here. Considering the curves for the plankton, it can be seen that there are certain points of correlation, assuming that the spring flowering occurred, as has been indicated earlier, before the beginning of February, and the summer one in the middle of August. When the phytoplankton was low, the salts began to accumulate. As the phytoplankton began to increase, the salts dropped off suddenly, implying that they were used up in the plant cells. The dying off of the phytoplankton was again followed by a gradual accumulation of salts. These were no doubt supplied in part by the decomposition of dead organic matter, and in part by new water entering the Sound. There are minor differences between the curves throughout, but in the major phases this general relationship holds. Nitrates seem to be somewhat more critical for phytoplankton in these particular waters than phosphates. There was always more PO_4 in the water column than NO_3, probably always enough for some plant growth. It is with the curve for NO_3 that the phytoplankton shows the greatest correlation, and it may be that only when the PO_4 curve agrees with that of NO_3 in trend that it can be considered related to the plankton production. There is a much more pronounced relationship between the phytoplankton and the salts at 2 meters and 15 meters than at the bottom, the 15-meter level showing the clearest correlation. The general ratio of nitrates to phosphates which is shown by Redfield (1934) to exist in the open oceans, and which corresponds in general to the proportions in which these occur in plankton organisms, does not hold at our station. Our data reveal that throughout most of the year the phosphates exceeded the nitrates, a condition which is contrary to that normally found.

Quantitatively, a greater number of organisms were found near the surface than at the bottom. The greatest development of phytoplankton is usually found to be in the lower strata of water, but at our particular station the amounts of detritus found in the water at all levels was so noticeably greater than that found in more open waters, that it is quite possible that throughout much of the year light is not available at the lower levels for photosynthesis. It happened very frequently that the number of cells at the bottom greatly exceeded that at 15 meters. This is probably explainable by the fact that cells sink rapidly once they become inactive, hence the active cells are those at the surface; those at the bottom have become inactive or are dead. In general the important species found at all three depths at any one time were the same. No counts obtained during the entire survey were high, the highest number recorded being 214,000 cells per liter at the surface in October, a number which is normally much exceeded during the spring maximum at other localities. Doubtless the numbers during the spring flowerings which occur in this region would exceed 200,000 many times. The lowest number recorded was 200 cells per liter at 30 meters in December, and at 15 meters in February. Collections made in March for the entire water column were lower than at any other time of year.

This survey, incomplete though it is, and lacking two important phases of the phytoplankton cycle, adds definitely to our knowledge of the waters near Woods Hole. It is the first study of the sort in this region of a station which is far enough from land to show some of the hydrographic features of the open sea. The quantitative work gives a reasonably accurate idea of the numbers of phytoplankton cells which occur in these waters at the different seasons; and the possible relationship between the plankton and the concentration of nitrates and phosphates is indicated.

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SERUM PROTEIN MEASUREMENTS IN THE LOWER VERTEBRATES. I. THE COLLOID OSMOTIC PRESSURE, NITROGEN CONTENT, AND REFRACTIVE INDEX OF TURTLE SERUM AND BODY FLUID

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The significance of the blood serum proteins in the maintenance of the fluid balance between blood and tissues was first suggested by Starling (1), who postulated that the osmotic pressure they exert is the force which, acting in opposition to the hydrostatic pressure of the blood, prevents the loss of excessive amounts of fluid to the tissues. The numerous studies of the serum colloid osmotic pressure which have since been made in man and the lower mammals have indicated that for mammals Starling's hypothesis holds true, if not completely, at least as a major factor. A comprehensive review of the subject, with bibliography, is that of Landis (2). A recent modification of method is that of Wies and Peters (3).

The question of the applicability of this hypothesis to conditions which obtain in the lower vertebrates has been investigated very little. Data on the amphibia are given by Landis (2) and also by Drinker and Field (4). The most recent work has been that of Keys and Hill (5), who studied six species of fish. It has seemed important to us to make a series of studies of the colloid osmotic pressure of the serum in several of the lower vertebrates, correlating the findings whenever possible with other determinations on the serum proteins. In this paper we present the results of a study of a reptile, the common "slider" turtle. Determinations were made of the colloid osmotic pressure, nitrogen content, and refractive index of the serum and of the body fluid of normal animals and of those subjected to long-continued starvation at two different temperatures.

METHODS

Fourteen adult, healthy, female turtles of the species *Malacoclemmys geographica*, Les. were obtained in October at about the end of the period of summer activity and feeding. Two of these were studied at once as normal fall animals and the remainder divided into four experimental groups, of which one was kept during the succeeding months under each of the following conditions: (1) room temperature, 19°–20° C., with feeding, (2) room temperature without feeding, (3) winter temperature, 7°–10° C., with feeding, (4) winter temperature without feeding. The food consisted of common garden worms *ad libitum;* all animals were kept constantly in large tanks of fresh water. During April at the end of the winter period of hibernation and fasting, two fresh specimens were obtained for use as spring controls.

Blood was drawn by syringe from the aortæ and sinus venosus and allowed to clot in tubes. After centrifuging, the supernatant serum was removed by pipette. A clear, colorless fluid which was frequently found in considerable amounts in the body cavity was removed by syringe without contamination by blood. It is this fluid which is referred to as body fluid.

Colloid osmotic pressures were obtained by the second method of Krogh and Nakazawa (6), using the modifications suggested by Turner (7). The membrane was Du Pont cellophane no. 450, the outer liquid 0.6 per cent sodium chloride. For every determination three or more osmometers were set up. The figure taken for the colloid osmotic pressure of any sample was the average of values given by osmometers which conformed to the requirements of the Krogh technique. Determinations of the total and non-protein nitrogen concentrations were made by the micro-Kjeldahl method combined with direct Nesslerization as given by Peters and Van Slyke (8). The difference between total and non-protein nitrogen gave the concentration of protein nitrogen. Total refractive indices were determined by a Zeiss dipping refractometer. Whenever the quantity of serum available made it possible, a determination was made of the refractive index of an ultrafiltrate prepared by the use of a Thiessen ultrafiltration apparatus with cellophane 450 as membrane. The total refractive index minus that of the ultrafiltrate gives the refractive index of the colloid fraction.

RESULTS

The protein data on sera and body fluids are given in Table I. The serum colloid osmotic pressure in animals of the warm, fed group was within the range of the normal fall values for a period of three months. The progressive decrease in pressure observed in all the other groups may be explained as due to the effects of starvation modified by the metabolic rates of the animals. In the warm, starved group total starvation plus a relatively high metabolic rate resulted in marked and immediate changes in the serum proteins as shown by the lowered colloid osmotic pressure. Edema was expected in these animals, but was not found either in this or in any other group. Since the turtles in the cold, fed group ate very little, both cold groups may be regarded as starved. Due apparently to the depression of the metabolism by the low temperature, the colloid osmotic pressure in these animals decreased more slowly than in the warm, starved group and the total fall was less. That the rate of metabolism in animals when subjected to different environmental conditions is so affected was shown by Hand (9) in his compilation of the results of inanition on the higher groups of animals.

TABLE I

Protein data from blood serum and body fluid, *Malacoclemmys geographica.* Body fluid was found only in the animals indicated. Protein nitrogen was obtained by subtracting the analytically determined non-protein nitrogen from the total nitrogen. The refractive index of the colloid fraction was obtained by subtracting the refractive index of the ultrafiltrate from the refractive index of the whole serum. An asterisk after the colloid refractive index indicates that in this case no ultrafiltrate was available. The figure was obtained as stated in the text.

Status of animal	Date killed	Length of experimental period	Colloid osmotic pressure		Total nitrogen		Protein nitrogen		Refractive index		
			Serum	Body fluid	Serum	Body fluid	Serum	Body fluid	Serum	Colloid fraction	Body fluid
		months	*mm. H_2O*	*mm. H_2O*	*mgm./cc.*	*mgm./cc.*	*mgm./cc.*	*mgm./cc.*			
Normal, fall...	Oct. 30	0.0	81	81	—	—	—	—	1.34350	.00768	1.33586
" " ...	Nov. 7	0.0	112	—	—	—	—	—	1.34453	.00886*	—
Warm, fed....	Jan. 4	2.0	98	46	6.54	0.75	6.29	0.61	1.34364	.00799	1.33586
" "	Feb. 2	3.0	99	—	6.83	—	6.60	—	1.34399	.00809	—
Warm, starved	Dec. 21	1.75	58	—	6.24	—	6.02	—	1.34508	.00907	—
" "	Jan. 17	2.5	58	28	5.85	0.83	5.45	0.51	1.34291	.00758	1.33632
" "	Mar. 1	4.0	39	36	6.58	0.79	6.34	0.58	1.34342	.00779	1.33636
Cold, fed......	Dec. 14	1.5	94	50	5.64	1.77	5.51	1.65	1.34341	.00773	1.33659
" "	Jan. 15	2.5	77	—	6.95	—	6.73	—	1.34474	.00907*	—
" "	Feb. 12	3.5	72	—	6.14	—	6.04	—	1.34339	.00766	—
Cold, starved..	Dec. 11	1.3	102	65	5.37	0.66	5.19	0.54	1.34356	.00789*	1.33604
" " ..	Jan. 10	2.3	57	41	4.70	1.23	4.55	1.07	1.34291	.00724*	1.33519
" " ..	Jan. 24	2.7	58	34	6.51	1.23	6.27	1.00	1.34350	.00760	1.33580
" " ..	Feb. 7	3.25	66	—	5.74	—	5.56	—	1.34548	.00981*	—
Normal, spring	Apr. 7	0.0	68	—	4.18	—	4.02	—	1.34208	.00671	—
" "	Apr. 9	0.0	48	—	5.11	—	4.90	—	1.34235	.00699	—

The occurrence of amounts of body fluid adequate for determinations was irregular and not limited to any group or groups nor to any particular time in the experimental period. Although the variations in the colloid osmotic pressure were wide, the value in each case was found to be lower than that of the corresponding serum except for one instance of equality. No proportional relation between the two pressures was discernible.

Table I shows total nitrogen and protein nitrogen figures, the latter obtained by subtracting the non-protein nitrogen from the total nitrogen. The non-protein nitrogen content was low and varied irregularly in the sera from .10 to .40 mgm./cc., with eleven of the fourteen values between .15 and .25 mgm./cc. In the seven analyses of body fluid the variation of non-protein nitrogen was from .12 to .32 mgm./cc. The concentration of protein varied widely yet it seemed clear that starvation in this species fails to cause a fall in the amount of protein parallel to the fall in colloid osmotic pressure. Table II gives the values in percentages, thus showing the lack of correspondence between the effect of starvation on colloid osmotic pressure and on protein content. The average of the two fall specimens is taken as 100 per cent for the colloid osmotic pressure series. It will be noted that the warm, fed animals show no fall. Nitrogen concentrations

TABLE II

A table showing *percentage* changes as the experiment progressed. The two normal fall animals are taken as 100 per cent. In the case of the nitrogen values, since no analyses were made on the fall animals, the 100 per cent is furnished by the two warm, fed animals whose other findings showed a negligible decrease as the months went by. The two spring animals were fresh from the collector, after a hibernation period of unknown duration probably much longer than the duration of the experiment.

Status of animal	Length of experimental period	Colloid osmotic pressure	Protein nitrogen	Refractive index
	months	*per cent*	*per cent*	*per cent*
Normal, fall	0.0	100	—	100
Warm, fed	2.0	101.5	100	96.5
" "	3.0	102.5	100	98
Warm, starved	1.75	60	93.5	110
" "	2.5	60	84.5	92
" "	4.0	40.5	98	94
Cold, fed	1.5	97.5	85.5	93.5
" "	2.5	80	104	110
" "	3.5	75	94	93
Cold, starved	1.3	106	80.5	95.5
" "	2.3	59	70.5	87.5
" "	2.7	60	97	92
" "	3.25	68.5	86	118
Normal, spring	—	70.5	62	81
" "	—	50	75.5	84.5

were not determined for the two fall animals but in the absence of that standard it seemed permissible to use the level of the two warm, fed animals as 100 per cent for protein comparisons. Determinations of total protein, albumin, and globulin in the sera of mammals have been reported by several observers (10, 11, 12, 13, 14) who have shown that during starvation a reversal of the mammalian albumin-globulin ratio occurs, indicative of a relatively higher globulin concentration. Because of the larger size of the globulin molecules this shift results in a marked fall in colloid osmotic pressure without a corresponding decrease in total protein concentration. From our observations it seems possible that a similar shift in albumin-globulin relations may occur in the turtle. The two spring specimens which had doubtless spent the entire winter in hibernation showed a fall in total protein more nearly approaching that in colloid osmotic pressure. The concentration of protein in the body fluid was much lower than in the serum and showed wide variations. No direct correlation with the colloid osmotic pressure was apparent.

The total refractive index of normal, fall turtles averaged 1.34377. With two exceptions the figures for animals with lowered colloid osmotic pressure were below this average, falling even to 1.34222 as an average for the two spring controls. The range of the refractive indices for the ultrafiltrates was narrow, with an average of 1.33569. Where no ultrafiltrate could be prepared, this average figure was used to calculate the probable value for the colloid fraction. (See Table I.) When the refractive index of the colloid fractions was plotted against the colloid osmotic pressure a good correspondence was evident, though there is a closer correspondence between protein concentration and refractive index of colloid fractions. The refractive index of the body fluid was consistently lower than that for the corresponding serum and higher than that of the serum ultrafiltrate. Since the crystalloids of serum and body fluid are probably closely alike, this difference between body fluid and ultrafiltrate from serum would indicate a colloid fraction in the body fluid, as verified by analysis.

DISCUSSION

Our interest has centered upon the inferences which may be drawn from these determinations as to the significance of the serum proteins. Certain points stand out: first, the fall in colloid osmotic pressure with prolonged starvation, especially at the higher temperature; second, the much greater stability shown during starvation in the quantity of serum protein as compared with its colloid osmotic pressure; third, the presence in the body fluid of substances exerting a colloid osmotic pressure far from negligible though the protein percentage is low. While the relation of plasma and true tissue fluid—meaning by this the fluid which has just made its exit through capillary walls—may not be that shown by the comparison of the serum and body fluid, it is nevertheless probable that capillary permeability to proteins, at least those of the smaller molecular size, is indicated by the very perceptible colloid osmotic pressure of the body fluid. Such proteins as may be outside capillary walls will, of course, lessen the effective colloid osmotic pressure of the plasma. Further, the marked decline of the colloid osmotic pressure in starvation without evident edema speaks against it as a major controlling factor in water balance between blood and tissues in this species, while the persistence of the protein content of the serum at a relatively high level may indicate the importance of plasma proteins for some other function than the maintenance of colloid osmotic pressure, possibly as nutritional reserves.

Summary

The average serum colloid osmotic pressure of two normal fall turtles of the species *Malacoclemmys geographica*, Les. was 96 mm. water pressure. This pressure was lowered by starvation, especially at laboratory as compared with winter temperature, but the serum protein concentration was much less affected. A body fluid obtainable from about half of the animals showed a colloid osmotic pressure of more than half the serum pressure in these individuals, though the protein content was low. These findings are interpreted as indicating that in this species the colloid osmotic pressure may not be the only controlling factor in the water balance between blood and tissues, and that other functions of plasma proteins may be of importance.

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SERUM PROTEIN MEASUREMENTS IN THE LOWER VERTEBRATES. II. IN MARINE TELEOSTS AND ELASMOBRANCHS

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The reasons for studying the plasma proteins of the lower vertebrates as shown in the first paper of this series (1) have to do with the validity of Starling's hypothesis for these forms and with the possible relation of the protein fraction of the plasma to nutritional needs. References to contributions in this field relating to the higher vertebrates will not be repeated. For the lower vertebrates, the list given by Drinker and Field (2) summarizes the information available up to their date of publication. A brief paper by Keys and Hill (3) gives the only findings for the colloid osmotic pressure of fish sera except those in two preliminary notes to this paper (4, 5). Interesting data allied in various ways are to be found in references (6) to (15) inclusive. The last two papers (14, 15) have excellent bibliographies.

The serum of various species of marine teleosts and elasmobranchs was studied at the Woods Hole Oceanographic Institution during the summers of 1933 and 1934, and during the summers of 1935 and 1936 at the Bergens Museum Biologiske Stasjon on the island of Herdla off the west coast of Norway. To both of these institutions most sincere thanks are due for the assistance generously rendered by them. A grant in 1936 from the Tracy McGregor Fund helped to defray expenses in that year.[2]

The determinations made were as follows: (1) colloid osmotic pressure, on all samples; (2) refractive index of the whole serum, on all samples; (3) refractive index on an ultrafiltrate, on many samples of the Norwegian series, whenever quantity of serum permitted; (4) total nitrogen and non-protein nitrogen in 1934, 1935, and 1936, a considerable number of determinations, whenever quantity permitted. In all, 121 individual samples representing 19 species of teleosts were studied and 48 individual samples from 11 species of elasmobranchs. A few of these samples were assembled from two or more small fish each. There were in addition some scattered determinations on other species.

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[1] Contribution No. 148.

[2] The nitrogen determinations of the Woods Hole series were made by Catherine Goffin and those of the Norwegian series by Cand. mag. Francis Wolff, of Bergens Museum. Acknowledgment is made of the careful and helpful work done by both. At Woods Hole the Marine Biological Laboratory made its facilities available to Miss Goffin for her work; at Bergen the Norwegian Fiskeriforsöksstasjon allowed the use of its equipment for cold storage and for chemical work. Without this help it would have been impossible to make the nitrogen analyses.

METHODS

Obtaining the Fish

The fish whose blood is to be studied should be in a healthy condition and must be uninjured, to prevent access of the surrounding medium to the blood. To secure fish that meet these standards is difficult. The ordinary methods of fishing are extremely rough when viewed from the standpoint of obtaining true samples of the circulating blood. The effect of the severe struggling often incident to capture may not be negligible. Grafflin (8) has told in detail of his care in this matter of reliable sampling. The fish included in this study have been progressively better and none are included in the figures reported about which there is appreciable doubt. Two alternatives present themselves. Blood may be drawn the moment the fish is taken and this is for some species, for example those from deep water which will not live in tanks, the only possible method; or the fish may be allowed to rest for some hours or longer in cars or tanks of water suitable in temperature and aeration. Feeding is possible in some cases where the fish live well in captivity. The effect of the initial struggling is thus eliminated and the state of the fish is steadier. At least four of the most consistent sets of determinations in this study have been made mainly on fish thus treated, *Opsanus tau*, *Tautoga onitis*, *Anarhichas lupus*, and *Raia erinacea.* In certain species the skin is so delicate that it is probably best not to use them in such studies as these unless unusual care can be taken. It is possible that the two pleuronectids in the Woods Hole series belong in this group.

Enough is known about the relation existing between the blood and tissues of fishes and the demands of the breeding season to make avoidance of this period desirable in a preliminary survey. However, the length of this season is often far beyond the months mentioned in books so that it has not always been possible to avoid it without sacrificing the use of fish otherwise very suitable. The depression in the breeding season well-known to trout hatchery men, when the fish will eat nothing or very little, is of indeterminate length and not easily avoided. The breeding fish used in this series are so described. When it is not mentioned that a species was apparently ripe it is to be understood that as far as known the samples were not from breeding fish.

Blood Sampling

The blood was taken in 1933 by syringe from gill vessels or heart; in the other three summers, in most cases from tail vessels, occasionally from the heart. For the suggestion of taking blood from the tail, the author is indebted to Dr. Homer W. Smith. The fish is wrapped in a cloth, laid in a deeply grooved board, ventral side up, restrained by lacing tapes across the board, and held by an assistant. The syringe needle is inserted in the mid-ventral line, somewhat behind the anus, often along the anal fin, at an angle which will take it between the hæmal spines. It is pushed in until the vertebral column is felt, is slightly withdrawn and pushed in again quickly, with a good chance of puncturing a tail vessel from which blood can be withdrawn with a minimum of injury and a maximum of ease. If the tail vessels are too small for use or are covered by too firm a membrane between the spines, the heart is exposed, the ventral aorta clamped off by a hemostat, and blood withdrawn from the bulbus or venous sinuses. The blood must be handled gently when discharged from the syringe into the centrifuge tubes else hemolysis may occur. The same thing may happen if the vessel used is not entered quickly and neatly. Heparin has been used in both syringe and centrifuge tubes, but some clotting is usually evident after centrifuging, hence the term serum has been thought more appropriate than plasma.

The blood was centrifuged three or four hours after being withdrawn, not earlier because in that case delayed and repeated clotting sometimes occurs and is very troublesome if in osmometer tubes. After centrifuging, the serum was set up at once in the osmometers or kept in a very cold refrigerator until next day. Because it was impossible in Norway to have the nitrogen determinations made on the first day, various preservatives were tried, but all resulted in the appearance of a precipitate. Serum kept at about 0° C., on the other hand, remains clear for a period up to at least three weeks, and refractometric measurements show little or no alteration. At rather low room temperatures, 16°–19° C., osmometers containing teleost serum have held steady as long as three days. In the case of elasmobranch blood, the occasional rather warm intervals at Woods Hole, with room temperatures up to 25°, in a few instances caused the appearance of a thin whitish film on the membrane in the osmometer, but the colloid osmotic pressure in these cases seldom varied from the usual range. These films were not seen at the temperature of the Herdla laboratory, usually 15°–18° C.

A sterile technique was not attempted. The place where the needle was to be pushed through the skin was carefully wiped with cotton. Syringes, needles, centrifuge and transfer tubes, and all other parts of apparatus coming into contact with the serum were cleaned systematically as follows. They were washed thoroughly with water, with soap when needed; they were rinsed thoroughly first with tap water, then with distilled water, finally with 70 per cent alcohol; they were dried in an oven at 70°–80° C. Contamination was not apparent except in the elasmobranch samples of the Woods Hole series in the hottest weather.

In general, then, while the securing, care, and preservation of the blood have presented difficulties, the samples included in this report are considered reasonably good. However, it is to be said that the history of the individual fish is in many important points entirely unknown. Its age, except as indicated roughly by its size, its recent activity when the fish has been bled immediately on being taken, its nutritive condition except that it may or may not have food in the digestive tract, all these factors of importance are not known. A considerable range in findings is to be expected.

TABLE I

Protein determinations on serum of *Opsanus tau*, toadfish. Fish all of one lot, kept in aquarium up to 10 days, fed. One or two months after the breeding season. The length of the specimens varied from ten to twelve inches. Nos. 1 and 6 were females, the others males. C.O.P. Colloid osmotic pressure in mm. water pressure, determined from one or two osmometers. N-P. N. Non-protein nitrogen.

No.	Av. C.O.P.	Refractive index	Total N.	N-P. N.	Protein N.
	mm./water		*mg./cc.*	*mg./cc.*	*mg./cc.*
1	104	1.34401	—	—	—
2	119	1.34498	8.13	.866	7.26
3	76	1.34498	9.28	.809	8.47
4	100	1.34470	6.73	.494	6.24
5	95	1.34375	6.77	.497	6.27
6	127	1.34369	6.55	.419	6.13
7	125	1.34457	5.63	.423	5.21
8	111	1.34386	7.44	.368	7.07
9	97	1.34509	—	—	—
10	84	1.34468	—	—	—
Averages	*104*	*1.34443*	*7.22*	*.554*	*6.67*

The Identification of Species

The identification of species was made at Woods Hole by the use of Bigelow and Welsh (16) and in Norway by Otterström (17). In both places much help was received from various persons who know the fishes of the region. Especial mention should be made of the Director at the Herdla laboratory, Professor August Brinkmann, and his son, and of the Assistant-Director, Dr. Rustad.

Methods of Testing

Colloid osmotic pressures were determined by the method of Krogh and Nakazawa (18) somewhat modified (19). The membrane used was cellophane no. 450. Refractive indices were determined by the dipping refractometers of Bausch and Lomb and Zeiss on both whole serum and its ultrafiltrate. The latter was secured from samples of the Norwegian series by a Thiessen apparatus, with care as to uniformity of technique. Two cubic centimeters of serum were placed in the apparatus with cellophane no. 450 as membrane and subjected to a pressure of some three atmospheres for a period of 12 hours. At the end of this time a clear, limpid filtrate was obtained, always protein-free. The residue on the membrane at this time was small in amount and slimy in character. Tests for chloride and reducing sugar showed them to be present in the ultrafiltrate. It was assumed that the membrane was permeable to practically all the serum crystalloids.

TABLE II

Protein determinations on serum of *Anarhichas lupus*, wolf-fish, 1935. Blood taken a few hours or a day after fish was brought to laboratory. Not near the breeding season. The length of the specimens varied from 18 to 30 inches. C.O.P. Colloid osmotic pressure in mm. water pressure. Derived from two to four osmometers in all cases except where only one osmometer was used, Fish No. 7. N-P. N. Non-protein nitrogen.

No.	Av. C.O.P.	n_D Serum	n_D Ultra-filtrate	n_D Colloid fraction	Total N.	N-P. N.	Prot. N.
	mm./water				*mg./cc.*	*mg./cc.*	*mg./cc.*
1	119	1.34530	1.33532	0.00998	6.15	.25	5.90
2	178	1.35588	1.33609	0.01979	10.94	.51	10.43
3	147	1.35051	1.33609	0.01442	7.96	.52	7.44
4	152	1.35128	1.33636	0.01492	8.55	.62	7.93
5	145	1.34702	1.33545	0.01157	8.12	.71	7.41
6	153	1.34832	1.33551	0.01281	—	—	—
7	150	1.34415	1.33551	0.00864	5.82	.77	5.05
8	177	1.34984	1.33578	0.01406	8.30	.77	7.53
Averages	*153*	*1.34916*	*1.33576*	*0.01328*	*7.98*	*.69*	*7.38*

Nitrogen determinations were made in 1934 at Woods Hole by the micro-Kjeldahl method with direct Nesslerization. For the non-protein determinations the precipitant was trichloracetic acid. In

1935 and 1936 the micro-Kjeldahl method was by titration. The precipitant was metaphosphoric acid. All methods and reagents were suitably controlled and tested.

TABLE III

The colloid osmotic pressure of the sera of marine teleosts. Arranged in order of height of osmotic pressure, in two series.

Name of species	Number of individuals	C.O.P. Range	C.O.P. Average
		mm. water	*mm. water*
Woods Hole Series			
Sarda sarda, Bloch. Bonito	1	233	233
Echeneis naucrates, Lin. Remora*	1	216	216
Tautoga onitis, Lin. Tautog*	24	79–193	129
Paralichthys dentatus, Lin. Summer flounder			
Pseudopleuronectes Americanus, Wal. Winter flounder	6	79–180	120
Prionotus Carolinus, Lin. Common robin*	7	86–122	106
Prionotus strigatus, Cuv. & Val. Red-winged robin*	7	87–117	102
Opsanus tau, Lin. Toadfish	10	76–127	104
Norwegian Series			
Scomber scombrus, Lin. Mackerel	3 samples fr. 11 fish‡	164–212	198
Anguilla vulgaris, Tur. Eel	4 samples fr. 13 fish‡	167–204	189
Belone acus, Risso. Hornfish	2	162, 184	173
Anarhichas lupus, Lin. Wolf-fish	13	85–181	147
Brosmius brosme, Asc. Cusk†	8	105–210	139
Molva molva, Flem.†	4	108–177	139
Gadus morrhua, Lin. Cod	11	70–192	117
Gadus pollachius, Lin. Pollack	8 samples 3 fr. 6 fish‡	31–172	94
Cyclopterus lumpus, Lin. Lumpfish*	3	75–87	81
Lophius piscatorius, Lin. Goosefish	8	25–60	42
Gadus virens, Lin.	1 fr. 2 fish‡	40	40

* Breeding season.

† From deep trawl, 300–400 meters. Fish with swim-bladders protruding or burst and with organs extruded in some cases on arrival at surface. Blood samples taken immediately on board the collecting boat.

‡ Samples from more than one fish because of small size of samples available from single individuals. The refractive indices were found to be similar before samples were combined.

RESULTS

The results of the study are presented in a series of annotated tables. The determinations on teleosts are given first. To show the range of variation among individuals of a single species *Opsanus tau*, Lin., toadfish, was chosen from the Woods Hole series of 1934, Table I, and *Anarhichas lupus*, Lin., wolf-fish, Table II, from the Herdla series of 1935. The toadfish was chosen as a sluggish fish, much studied at Woods Hole laboratories, and the wolf-fish as a more vigorous species much studied in Norway.

TABLE IV

Refractive indices of sera and ultrafiltrates from marine teleosts. Arranged in order of height of C.O.P. Figures in parentheses indicate the number of individuals used for the following average, when this is less than the total number.

Name of species	Number of individuals	n_D Serum	n_D Ultrafiltrate	n_D Colloid
Woods Hole Series				
Sarda sarda	1	1.35220	—	—
Echeneis naucrates	2	1.35158	—	—
Tautoga onitis	24	1.34629	—	—
Paralichthys dentatus				
Pseudopleuronectes Americanus	6	1.34313	—	—
Prionotus Carolinus	7	1.34329	—	—
Prionotus strigatus	7	1.34205	—	—
Opsanus tau	10	1.34443	—	—
Norwegian Series				
Scomber scombrus	3 samples fr. 11 fish*	1.35000	(2)1.33690	0.01306
Anguilla vulgaris	4 samples fr. 13 fish*	1.35152	1.33552	0.01600
Belone acus	2	1.35084	1.33724†	0.01360
Anarhichas lupus	13	1.34852	1.33577	0.01275
Brosmius brosme	5	1.34703	1.33574	0.01169
Molva molva	4	1.34597	(2)1.33601	0.00912
Gadus morrhua	11	1.34421	1.33588	0.00834
Gadus pollachius	8 3 of these fr. 6 fish*	1.34401	1.33594	0.00807
Cyclopterus lumpus	3	1.34388	1.33535	0.00853
Lophius piscatorius	8	1.34168	1.33545	0.00623
Gadus virens	1 sample fr. 2 fish*	1.34158	1.33559	0.00599

* See note on Table III.

† Samples used for obtaining ultrafiltrates in these cases were only one cubic centimeter instead of two as usual. This may explain the aberrant value.

Table III summarizes the data on colloid osmotic pressure from the teleosts of Woods Hole and Herdla; Table IV, the data on refractive indices; Table V, the nitrogen determinations. The fish from Woods Hole and Herdla are listed separately, since the procedures were more complete in the later series. The order of names in all tables is that of the relative heights of the colloid osmotic pressure, even though the order of refractive indices and of nitrogen content does not follow exactly the same course.

In a similar manner Tables VI–IX present the data for elasmobranchs, Table VI showing the variation among individuals of a single species, *Raia erinacea*, Lin., small skate, and Tables VII–IX the colloid osmotic pressure, refractive indices, and nitrogen findings respectively for the several species of elasmobranchs.

An effort was made to study *Myxine glutinosa*, Lin., hagfish, but it was found very difficult to secure blood samples entirely uncontaminated by slime. The individuals were not large enough to yield more than about one cubic centimeter of blood, often less, and therefore of necessity the determinations were made on mixed samples, though the refractive indices were taken before mixing and only samples of similar indices combined. From one such mixed sample two osmometers gave an average of 24 mm. c.o.p., the total serum showed a n_D of 1.35073, the ultrafiltrate n_D 1.33923, thus giving a colloid index of 0.01150. The total N of the serum was 5.88 mgm./cc.

TABLE V

Nitrogen determinations on sera of marine teleosts.

Name of species	Number of individuals	Total N.	N-P. N.	Protein N.
		mg./cc.	*mg./cc.*	*mg./cc.*
Woods Hole Series				
Echeneis naucrates	1	6.68	.272	6.41
Tautoga onitis	6	6.62	.498	6.12
Paralichthys dentatus				
Pseudopleuronectes Americanus	3	5.48	.452	5.03
Prionotus Carolinus	1	7.90	.747	7.15
Opsanus tau	7	7.22	.554	6.67
Norwegian Series				
Scomber scombrus	2 samples fr. 9 fish*	8.30	.65	7.66
Anguilla vulgaris	3 samples fr. 11 fish*	8.47	1.19	7.28
Anarhichas lupus	10	8.11	.70	7.48
Brosmius brosme	1	6.95	.57	6.38
Gadus morrhua	5	5.93	.60	5.33
Gadus pollachius	3 samples fr. 5 fish*	6.11	.76	5.34
Cyclopterus lumpus	3	5.37	.25	5.09
Lophius piscatorius	8	4.85	.44	4.41
Gadus virens	1 sample fr. 2 fish*	3.99	.39	3.60

* See Table III.

TABLE VI

Protein determinations on serum of elasmobranch, *Raia erinacea*, small skate. Fish of different lots, kept in aquarium not more than three or four days. Height of breeding season, several eggs laid in aquarium. The length of the specimens varied from 17 to 19 inches. Nos. 2, 3 and 9 were males, the rest females.

C.O.P. Colloid osmotic pressure, determined from one to three osmometers, usually two.

N-P. N. Non-protein nitrogen.

No.	Av. C.O.P.	Refractive index	Total N.	N-P. N.	Protein N.
	mm./water		*mg./cc.*	*mg./cc.*	*mg./cc.*
1	63	1.34694	16.83	—	—
2	73	1.34886	16.95	—	—
3	14	1.34665	—	—	—
4	50	1.34581	—	—	—
5	47	1.34895	—	—	—
6	71	1.34765	—	—	—
7	17	1.34661	15.91	—	—
8	16	1.34661	14.78	13.55	1.23
9	14	1.34849	13.13	13.02	0.11
10	34	1.34683	16.39	13.19	3.20
11	19	1.34618	16.39	13.71	2.68
12	29	1.34640	16.75	13.88	2.87
Averages	*37*	*1.34715*	*15.49*†	*13.47*†	*2.02*†

† Five only used for average.

TABLE VII

The colloid osmotic pressure of the sera of elasmobranchs, arranged in the order of the height of the osmotic pressures.

C.O.P. Colloid osmotic pressure.

Name of species	Number of individuals	C.O.P. Range	C.O.P. Average
		mm./water	*mm./water*
Woods Hole Series			
Carcharias taurus, Raf. (sp.?) Shark	1	46	46
Mustelus canis, Mitch.† Smooth Dogfish	4	33–58	41
Raia erinacea, Mitch.† Small Skate	12	14–73	37
Dasybatus marinus, Klein. Stingaree	1	36	36
Norwegian Series			
Raia fullonica, Lin.‡	2	14, 49	32
Chimæra monstrosa, Lin.‡	5	10–40	27
Galeus vulgaris, Flem. Shark	1	27	27
Squalus acanthias, Lin.‡ Spiny Dogfish	2	23, 31	27
Pristiurus catulus, Gun.‡	7	5–59	26
Raia oxyrhynchus, Lin.† ‡	6	11–45	22
Etmopteris spinax, Lin.† ‡	7 samples fr. 14 fish*	−8–34	17

* Individual fish small, about 12–14 inches. Samples were therefore combined, but only after refractive indices had shown them to be in the same range. Two negative values may indicate the error of the method when the pressure is extremely low.

† Breeding season.

‡ From deep trawl, 300–400 meters. No outward sign of injury on coming to the surface. *E. spinax* and *R. fullonica* lived often as long as 24 hours in laboratory tanks.

Table VIII

Refractive indices of sera and ultrafiltrates from elasmobranchs. The number in parentheses (4) indicates that the following n_D was derived from 4 individuals only. See notes on Table VII.

Name of species	Number of individuals	n_D Serum	n_D Ultra-filtrate	n_D Colloid
Woods Hole Series				
Carcharias taurus	1	1.34601	—	—
Mustelus canis†	4	1.34618	—	—
Raia erinacea†	12	1.34715	—	—
Dasybatus marinus	1	1.34682	—	—
Norwegian Series				
Raia fullonica‡	2	1.34852	1.34079	0.00774
Chimæra monstrosa‡	5	1.34596	(4) 1.34063	0.00486
Galeus vulgaris	1	1.34422	1.34113	0.00309
Squalus acanthias‡	1	1.34671	1.34096	0.00575
Pristiurus catulus‡	7	1.34423	1.34076	0.00331
Raia oxyrhynchus† ‡	5	1.34412	1.34070	0.00342
Etmopteris spinax† ‡	7 samples fr. 14 fish*	1.34353	1.34055	0.00288

the N-P.N., 0.22 mgm./cc. These figures do not seem concordant, but are reported since data on this form are few.

Body fluid was present in one instance only in quantity sufficient for collection and study. This was in a wolf-fish of medium size and

Table IX

Nitrogen determinations on sera of elasmobranchs. See notes on Table VII. Number in parentheses (1) indicates that in only one instance was there serum enough for the determination of the N-P. N.

Name of species	Number of individuals	Total N	N-P. N.	Protein N.
		mg./cc.	*mg./cc.*	*mg./cc.*
Woods Hole Series				
Raia erinacea†	5	15.49	13.47	2.02
Dasybatus marinus	1	16.85	11.81	5.04
Norwegian Series				
Raia fullonica‡	3	17.50	12.16	5.34
Chimæra monstrosa‡	1	13.91	11.15	2.76
Galeus vulgaris	1	13.76	11.47	2.29
Squalus acanthias‡	1	14.98	12.68	2.30
Pristiurus catulus‡	3	12.72	9.58	3.14
Raia oxyrhynchus† ‡	6	15.07	12.31	2.76
Etmopteris spinax† ‡	4 samples fr. 5 fish*	12.18	(1) 10.07	1.60

medium serum values. There was nothing to indicate that the fish was not in a healthy condition. The data are given in Table X. The body fluid showed a c.o.p. nearly four-fifths that of the serum, and while the refractive index did not show quite as high a colloid fraction, yet it is clear that this fluid contained a considerable amount of protein, an amount comparable to that found in the body fluid of the "slider" turtle (1).

Discussion of Results

Regarding the results on teleosts, it is apparent that the findings for a single species show a range proportionately wider that we expect in mammals. Such a lack of constancy has been found by other workers on fish blood. (See references in the first paragraph of this paper.) Whether this is really true or due, in spite of efforts to the contrary, to the use of unsuitable specimens is not known. Studies are in progress on fresh-water species living in the controlled environment of fish hatchery ponds to see if the variation from individual to individual is as great as that seen in the toadfish, 76–127 mm. or in the wolf-fish, 119–178 mm. water pressure. Also the yearly rhythm is being followed to see whether colloid osmotic pressure shows the effect of elaboration of sex products and of spawning depression.

Table X

Comparison of serum and body fluid from one specimen of *Anarhichas lupus* from which enough body fluid was obtainable for colloid osmotic pressure and refractometer tests though not for nitrogen analyses.

Fluid	C.O.P.	n_D Serum or fluid	n_D Ultrafiltrate	n_D Colloid
	mm./water			
Blood serum..........	129	1.34707	1.33563	0.01144
Body fluid...........	98	1.34184	1.33532	0.00652

There is obviously a wide difference between the colloid osmotic pressure of the goosefish, 42 mm. and that of the mackerel, 198 mm. This latter value is similar to that found by Keys and Hill (3) for *Anguilla*, the European eel, and verified in this series. The ranges for individuals of these species are seen to be mutually exclusive, but those for species nearer the middle of the total range overlap freely and some are indistinguishable, as for instance the two species of *Prionotus*. To what this characteristic range for a species is related is not known though various suggestions have been made. Arterial pressure determinations on fish are few and reliable determinations still fewer (20), while determinations of capillary pressure have not been made at all. The degree of habitual activity has been suggested as a clue, for *Lophius* is proverbially sluggish and *Scomber* very active, while the extensive migrations of *Anguilla* are well-known. The series is as

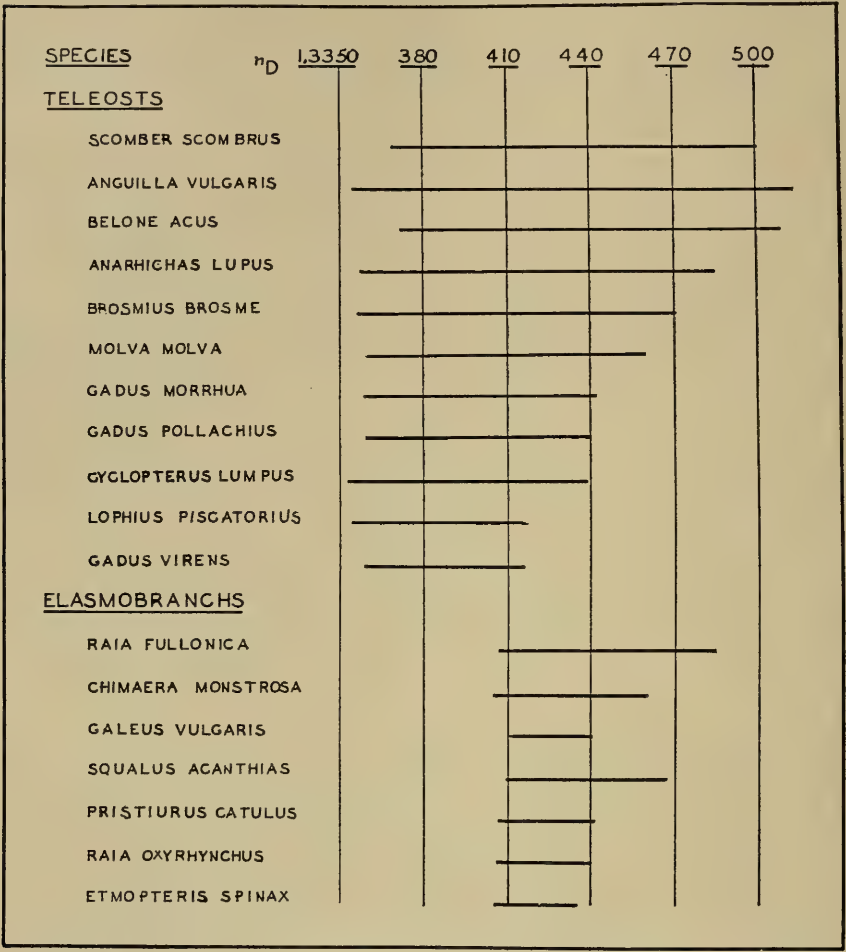

Chart 1. Refractive indices of colloid fraction in Norwegian series of teleosts and elasmobranchs. The left end of each line shows the refractive index of the ultrafiltrate, the right end that of the whole serum. The length of the line therefore shows the index of the colloid fraction. The ultrafiltrates in all of the teleosts except two are closely grouped, indicating relative constancy in the non-colloid components of the sera. The two exceptions are in fish which were represented by limited samples only, in the case of Belone by samples for ultrafiltration of only half the usual size. The high position of the ultrafiltrate index for all elasmobranchs is related to their high non-protein nitrogen fraction.

yet too short to give an answer here. The possibility of other substances which may add to the colloid osmotic pressure of the serum, e.g. fats (21, 22) is not to be excluded particularly when one occasionally sees globules of fat at the top of a centrifuged tube of blood and infers the presence of associated substances in solution in appreciable amounts. Also the wide variation in the fat content of fish is a matter of common knowledge. No determinations on serum fats have been made. The albumin-globulin ratios in fish are said by Lepkovsky (11) to vary widely. Such variations, with their attendant contrast in the size of the protein molecules, may help to explain not only differences between species but irregularities in the relationships between colloid osmotic pressure and other protein data. A few preliminary studies on plasma protein regeneration after depletion by

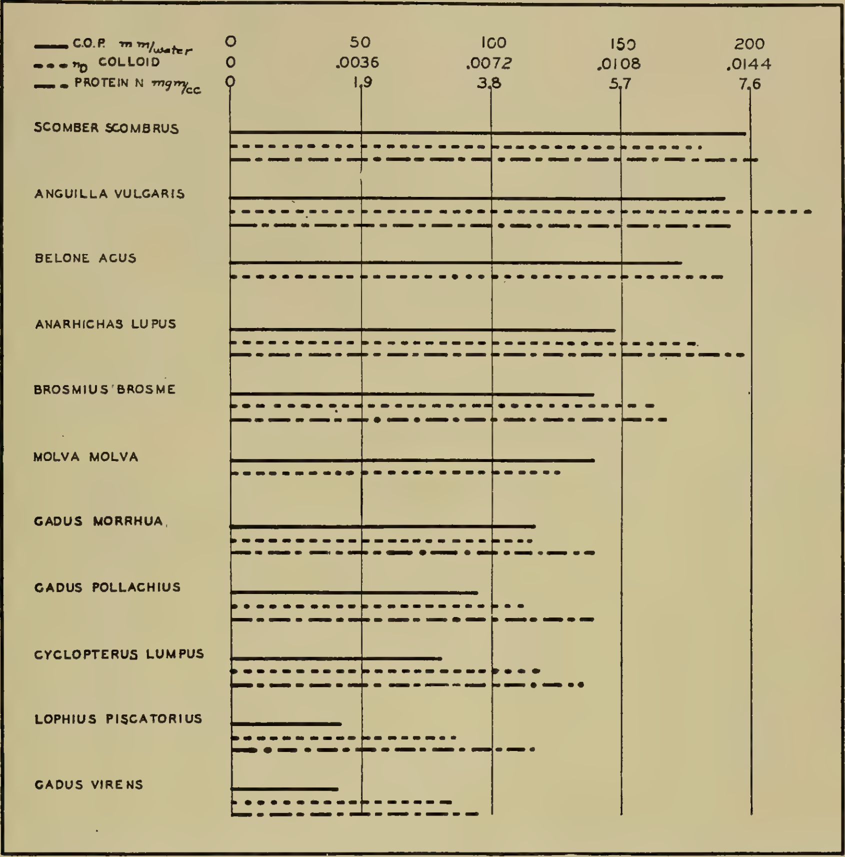

CHART 2. Comparison of colloid osmotic pressure, refractive index of colloid fraction, and protein nitrogen in Norwegian teleost series. The several ranges have been adjusted so that the maximum values are shown by lines of approximately equal length. The relative values of the three measurements thus appear, with a range much wider in c.o.p. than in n_D or protein N. This is probably to be explained by a variation in the albumin-globulin ratio in the direction of large molecules when the total quantity is small.

hemorrhage seem to indicate that protein may come back rather quickly, perhaps more quickly in total quantity than in colloid osmotic pressure, which may be the same as saying that the globulins regenerate—or are gotten into the blood—more rapidly than the albumins. This obviously suggests the experiments on mammalian protein regeneration from the laboratories of Whipple and Weech, to whose papers only partial references are given (23, 24, 25).

As the refractive indices are scrutinized, it is apparent that the range for the ultrafiltrates is narrow, indicating constancy in the non-protein fraction of the plasma, while the refractive index for the colloid fraction varies widely from species to species and accompanies the c.o.p. though the correspondence is not too close. (See Chart 1.) The analyses for total and non-protein nitrogen similarly give values for the serum proteins which vary approximately with the c.o.p. but follow more closely the refractive indices of the colloid fractions. These correspondences and differences are brought out in Chart 2. It is to be noted that the range of c.o.p. from the species of low to those of high values is much greater than the range in protein content. This fact may be associated with the ready appearance of protein molecules of large size mentioned above. It is possible that in the life economy of teleosts it is of especial importance to hold the total protein content of the serum at a definite level while the assortment of proteins may vary. It would follow from this that a rigid maintenance of colloid osmotic pressure is impossible or unnecessary.

For the elasmobranchs it is to be said that all averages for colloid osmotic pressure are low, though occasional individuals have been found as high as 70 mm. water pressure. As expected, the total and non-protein nitrogen findings, the refractive indices for total sera and for ultrafiltrates are all high, reflecting the high urea or trimethylamine oxide content of elasmobranch blood. The protein nitrogen figures are low with two exceptions, one specimen of *Dasybatus marinus*, stingray, and three of *Raia fullonica*, a Norwegian skate. Differences in the physiological make-up of elasmobranchs and teleosts thus include the protein blood picture.

Summary and Conclusions

1. In a study of 121 individuals of 19 species of marine teleosts it has been found that the range of colloid osmotic pressure shown by the blood serum is wide for a single species; that the c.o.p. shows a characteristic range in each species; and that for the species studied the c.o.p. varies from an average of 42 mm. water pressure in *Lophius piscatorius*, Lin., goosefish, to about 200 mm. in *Scomber scombrus*,

Lin., mackerel, *Anguilla vulgaris*, Tur., eel, and probably others. Intermediate values for averages within species were found most commonly, from about 100 to 150 mm.

2. The refractive indices for the colloid fractions of the sera and the protein nitrogen values follow the same general distribution as the c.o.p. though correspondence is not perfect. The c.o.p. seems to vary through a much wider range than the protein N or the refractive index of the colloid fraction.

3. All protein values for the 48 individuals of 11 species of elasmobranchs were found to be low, distinctly lower than in all save the very lowest of the teleosts.

4. The refractive indices of the ultrafiltrates from the sera in all teleosts were very constant as compared with the wide range of the colloid figures. This constancy, reflecting the crystalloid status of the sera, is also characteristic of the elasmobranch ultrafiltrates though the absolute level is materially higher in the latter group, a fact associated obviously with the high non-protein nitrogen content of elasmobranch blood.

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GENETICS AND HISTOLOGY OF THE COLOR PATTERN IN THE NORMAL AND ALBINO PARADISE FISH, MACROPODUS OPERCULARIS L.[1]

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The paradise fish is well known to fish fanciers. It is thought to have been the first "fancy" tropical fish to be bred in Europe, having been first introduced in Paris by Carbonier in 1868. Specimens of the albino variety utilized in this investigation were obtained from dealers in New York City during the fall of 1934. These were at that time a novelty and it is stated by Innes (1935) that they were first imported from German fanciers in 1933.

The paradise fish is one of the labyrinth fishes and is referred to Order Labyrinthici, Family (368) Osphronemidæ in the classification by Jordan (1923). It is also known in the literature as *Macropodus viridi-auratus*. A description is given by Regan (1909). This fish is found in the lowland streams of China, Formosa and Cochin China. It is well fitted to live in stagnant waters because its accessory respiratory apparatus, the labyrinth organ, allows it to use atmospheric oxygen as well as to breathe by gills. On this account almost no attention needs to be given to aeration of water in an aquarium and it may be kept in very small containers.

The paradise fish is a "bubble nest" builder. The male, presumably by aid of some oral secretion, makes a floating structure of bubbles into which he shoots the eggs as they are laid by the female. The mating and spawning activities, in our experience, take place at temperatures ranging from 26° C. to 29° C. Certain fish dealers, however, state that they will breed at as low a temperature as 20° C. Studies made by Goodrich and Taylor (1934) on a species *Betta splendens*, a related genus, showed a remarkably precise limitation of the breeding activities to a temperature of about 26° C. It is necessary, under aquarium conditions, to remove the female after laying, as she may eat the eggs, and to remove the male after hatching as he may eat the young fish. The young fry are first fed from Paramecia cultures and later with *Daphnia*. The adults are fed with enchytraeids

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The senior author wishes to acknowledge his indebtedness to his assistant, Miss Priscilla Anderson, for carrying certain parts of this work to completion.

(the white worms of dealers), minced earthworms, liver and dry prepared fish foods. Further details of the care of the fish are given by Innes (1935). The albino variety is much more difficult to rear. It is less viable and takes food less readily. It seems to avoid light more than the dark variety and possibly for this reason it is less likely to discover food.

GENETIC EXPERIMENTS

The dark or normal colored paradise fish when crossed with the albino variety produced all dark type in the F_1 generation. The F_2 in two separate crosses gave the results shown in Table I. The results of the back cross between the F_1 and the albino are also shown in Table I. Similar results were independently obtained by Kosswig (1935) though with somewhat greater deviation from the expected ratios. Our counts were made eight days after spawning and before the onset of the high mortality which is unfortunately frequent in fish cultures. These results clearly indicate that the normal and albino types are a pair of mendelian allelomorphs in which the normal is dominant over the albino.

TABLE I

F_2 from cross between normal and albino varieties

Date of spawning	Number of dark	Number of albino	Ratio
9-18-35	204	62	3.29/1
1-21-36	438	145	3.02/1

F_1 from cross between heterozygous dark and albino

Date of spawning	Number of dark	Number of albino	Ratio
9-27-35	226	221	1.02/1
3-12-36	552	551	1.002/1

Counts in both cases made eight days after spawning.

THE COLOR PATTERN

The normal or wild type paradise fish (Fig. 1) is strikingly marked on the sides with vertical stripes which are referred to in this paper as green and red stripes. The color, however, varies with environmental conditions. The striping may largely disappear when the fish is placed against a white background. The green stripes may be more accurately described as being a metallic blue-green when viewed from an angle which strongly reflects light. From other angles these stripes may appear much darker, giving a mottled blue and black appearance. The red stripes are of an orange-red hue. The markings in the male are more brilliant than in the female, especially at the breeding period. The albino (Fig. 2) is light colored and has pink eyes but shows faint orange and blue-green stripes. As will be shown later in detail, and as has been mentioned by Kosswig (1935), the black pigment is entirely absent.

HISTOLOGY OF THE COLOR PATTERN

The cells involved in the production of the color pattern are the black cells or melanophores, yellow cells or xanthophores, red cells or erythrophores and those containing reflecting crystals or iridocytes. There is some evidence for considering that the erythrophores develop from the xanthophores in this fish. The xanthophores contain yellow pigment granules soluble in 95 per cent alcohol in about two hours. The erythrophores contain orange-red granules less readily soluble in alcohol but sometimes xanthophores are observed which contain a few granules similar to those in the erythrophores. The erythrophores appear later in development and old fish show more red pigment than young fish.

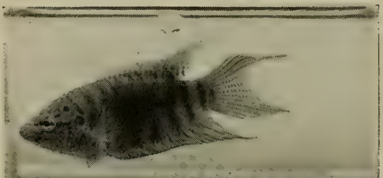

FIG. 1. Normal paradise fish.

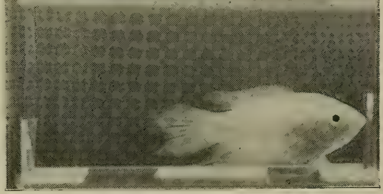

FIG. 2. Albino paradise fish. The pictures of the two fish were taken simultaneously in one aqarium.

We recognize three zones or areas of disposition of chromatophores in relation to the individual scales. All of these are probably dermal as they lie beneath the stratified epithelial layer of the epidermis. The relationships of these areas are illustrated by Figs. 3 and 4 which show diagrammatic reconstructions of median longitudinal sections of a series of scales taken perpendicular to the body surface. These diagrams are based upon microtome sections made by three freezing methods and upon dissections of the body wall made under a dissecting binocular microscope. The first or superficial zone (*a*) (Figs. 3 and 4) lies on the upper outer surface of the scale and is connected by the second and upward sloping intermediate zone (*b*) with the third or deep zone (*c*) located on the underside of the next anterior overlying scale. This deep zone also lies above a stratum of fat cells (*d*) which separates it from the inner upper surface of the underlying scale.

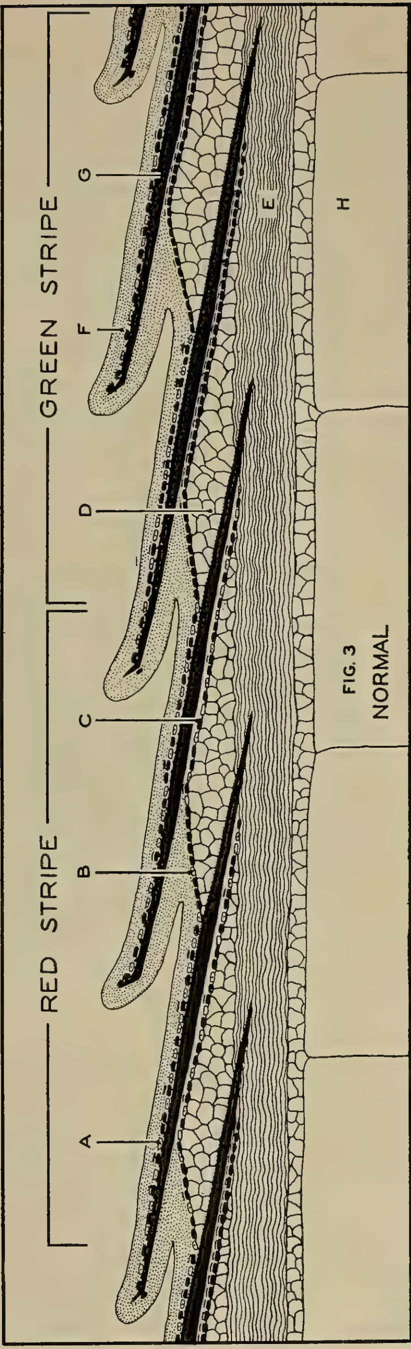
RED STRIPE
GREEN STRIPE
A
B
C
D
E
F
G
H

FIG. 3
NORMAL

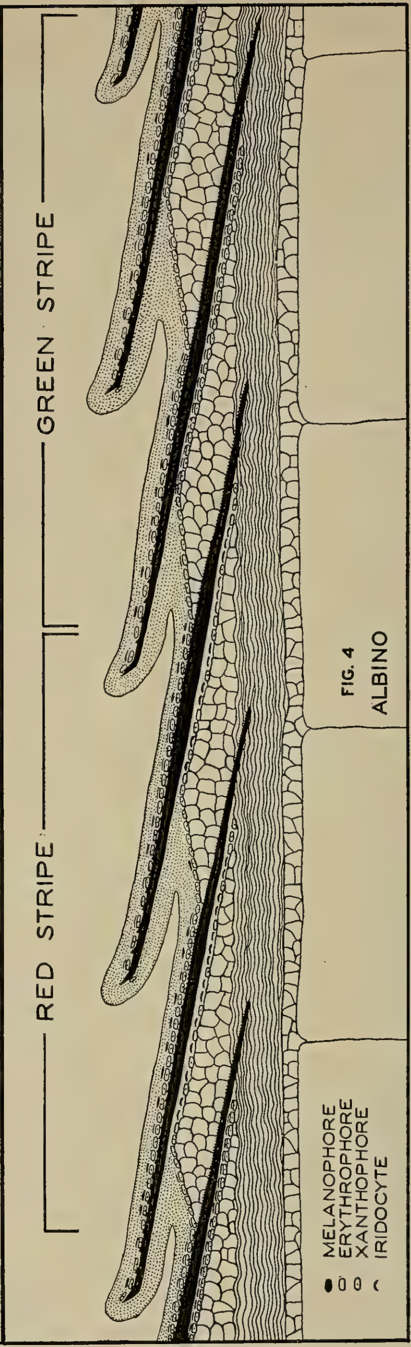
RED STRIPE
GREEN STRIPE
● MELANOPHORE
0 ERYTHROPHORE
0 XANTHOPHORE
(IRIDOCYTE

FIG. 4
ALBINO

FIG. 3. Normal dark fish. Diagram of median longitudinal section of a series of scales. Magnification × 80. (*A*) Superficial zone of chromatophores; (*B*) intermediate zone of chromatophores; (*C*) deep zone of chromatophores; (*D*) stratum of fat cells; (*E*) connective tissue layer; (*F*) stratified epithelium; (*G*) scale; (*H*) muscle. Chromatophores reduced to about 75 per cent of actual number for clarification of diagram.

FIG. 4. Albino fish. Details as in Fig. 3.

TABLE II

Dark-colored fish. Counts of chromatophores in area of 0.054 sq. mm. M, melanophores, X, xanthophores, E, erythrophores, I, iridocytes.

	Superficial zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	22	20	0	6	15	26	0	3	
	23	18	0	3	12	20	0	2	
	17	17	0	5	13	20	0	1	
	18	24	0	4	14	18	0	2	
	19	23	0	3	18	21	0	5	
	19	17	0	3	19	20	0	0	
	25	19	0	4	20	19	0	6	
	15	22	0	2	21	26	0	3	
	18	22	0	4	21	23	0	6	
	19	18	0	0	15	25	0	7	
Average first 10....	19.5	20	0	3.4	16.8	20.6	0	3.5	Total
Average first 20....	21.2	20.2	0	3.6	18.5	20.8	0	3.6	87.9
	Deep zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	43		0	34	19	11	3	4	
	42		0	40	19	5	5	2	
	38		0	36	17	9	3	2	
	25		0	25	15	3	3	0	
	36		0	36	18	5	3	1	
	30		0	20	17	14	3	3	
	27		0	27	18	8	5	0	
	28		0	28	15	15	3	1	
	35		0	35	18	9	3	3	
	37		0	37	14	5	3	3	
Average first 10....	34.1		0	31.8	17	8.5	3.4	1.9	Total
Average first 20....	34.1		0	32.5	18.5	7.7	3.6	2.8	99.2

Only the first ten of twenty counts are tabulated but the average of all twenty is given.

Counts of iridocytes have a greater possibility of error than that of other chromatophores because it is difficult to determine the boundaries of individual cells.

Counts of the xanthophores from the deep zone of the dark fish (Table II) are not tabulated because they were so covered by melanophores and iridocytes that no accurate count could be made, but they are few in number or absent.

In the dark or normal type of paradise fish (Fig. 3) the upper zone contains a fairly uniform distribution of melanophores, xanthophores and iridocytes. The iridocytes tend to be superficial to and often directly above melanophores in all zones. Table II shows the relative numbers of these cells in both the green and the red stripes. The counts in the table were made on scales removed from the body and treated with adrenalin to cause a concentration of pigment in the cells

Table III

Albino fish. Counts of chromatophores as in Table II.

	Superficial zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	0	20	0	14	0	30	0	16	
	0	27	0	13	0	28	0	19	
	0	26	0	17	0	30	0	14	
	0	29	0	16	0	31	0	17	
	0	25	0	14	0	31	0	18	
	0	27	0	18	0	29	0	10	
	0	23	0	10	0	26	0	14	
	0	24	0	11	0	30	0	15	
	0	28	0	14	0	23	0	10	
	0	31	0	17	0	24	0	19	
Average first 10....	0	26.8	0	14.4	0	28.2	0	15.2	Total
Average first 20....	0	27.4	0	16.4	0	27.6	0	16.1	87.5
	Deep zone								
	Green stripe				Red stripe				
	M	X	E	I	M	X	E	I	
	0	17	0	20	0	13	4	18	
	0	18	0	23	0	7	3	17	
	0	12	0	19	0	8	6	10	
	0	15	0	20	0	6	5	10	
	0	20	0	17	0	10	3	21	
	0	15	0	16	0	4	5	11	
	0	21	0	22	0	13	3	22	
	0	34	0	23	0	6	4	20	
	0	31	0	26	0	11	6	15	
	0	33	0	34	0	10	4	18	
Average first 10....	0	21.6	0	22.0	0	8.8	4.3	16.2	Total
Average first 20....	0	21.7	0	23.1	0	8.2	4.4	15.4	72.8

and so to help to distinguish one cell from another. The counts were of cells seen within one quadrant of an ocular counting disc. It is calculated that the area so outlined is 0.054 sq. mm. These counts will therefore when multiplied by the factor 18.4 give the approximate number of cells per square millimeter. It will be noticed that there is probably no significant difference between numbers of cells of the upper zone in the green and in the red stripes. It is the deep zone which provides the basis of the striping. Counts were made on the deep zone after removal of scales and after washing the side of the body with an adrenalin solution. It will be noted in Table II that in the deep zone the green stripes, in contrast with the red stripes, show a significant excess of melanophores and of iridocytes and but few or no xanthophores or erythrophores and that these latter are found in the red stripes. The intermediate zone very seldom contains any erythrophores and there are also fewer iridocytes than in the outer zone. It is much the same whether located in a green or a red stripe and therefore is, like the outer zone, neutral in regard to striping.

No melanophores are found in either stripe in the albino fish (Fig. 4 and Table III). Tests with adrenalin to concentrate pigment (*cf.* Goodrich, 1927) or use of the "dopa" reaction (*cf.* Goodrich, 1933) failed to indicate the presence of any "colorless" melanophores. Otherwise the disposition of cells is similar to that in the normal type except that there are more xanthophores and iridocytes.

Developmental History

The melanophores first appear at about the 16-somite stage (nineteen hours in our cultures). These are at first rather irregularly distributed and there is no indication of pattern formation until the fish is from nine to twelve days of age (about 3 mm). At this time from six to eight spots appear on both the mid-dorsal and mid-ventral aspects of the body and tail region. The striping first becomes visible in the 10 to 14 mm. fish. The number of stripes is comparable with the number of earlier spots but no certain relationship has been demonstrated.

Discussion

Histological studies on the light color phases of various fish have shown that different cellular complexes may produce these light types. In *Aplocheilus* (*Oryzias*) *latipes* a double recessive (Aida, 1921) owes its absence of color to a reduction of amount of melanin produced in melanophores and of xanthine in xanthophores (Goodrich, 1927). This circumstance in other animals has given rise to the term "colorless" chromatophores. In this case the cells seem incapable of producing the normal amount of chromogen although sufficient oxydase is present (Goodrich, 1933). In the goldfish it is found that the light type, the transparent shubunkin, owes its condition to the early disintegration of melanophores and erythrophores (Goodrich and Hansen, 1931). In the paradise fish as described above no melanophores of any type have been seen at any stage of development. This latter form is a true albino, as no pigment is present in the eyes in contrast with the other types named above. The senior author of this paper has unpublished observations on an albino trout which has no pigment in the eyes but does have melanophores with reduced pigment as in *Oryzias*. In each case among fish so far investigated in histological detail a different developmental mechanism is concerned in producing the light phase.

SUMMARY

1. In the paradise fish, *Macropodus opercularis*, the dark-colored or normal type is a Mendelian dominant to the albino.

2. A description is given of the cell groupings which form the basis of the color pattern.

3. The melanophores are entirely absent from the albino but all other types of chromatophores are present.

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CHROMATOPHORE REACTIONS IN THE NORMAL AND ALBINO PARADISE FISH, MACROPODUS OPERCULARIS L.

H. CLARK DALTON AND H. B. GOODRICH[1]

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The paradise fish, *Macropodus opercularis* L., was chosen for this study because there are two varieties, the normal dark type and the albino. It was thought that the albino would afford special opportunity for studying the erythrophores and xanthophores because these are not hidden by the melanophores, as they are in the dark type. These two varieties would then give a good basis for comparing the reactions of the three types of chromatophores.

The paradise fish is well known to fish fanciers. Its native habitat is the shallow coastal streams of southeastern Asia. Details in regard to the characteristics and care of this fish may be found in the book on exotic aquarium fish by Innes (1935). The albino variety apparently was first imported into this country from Germany in 1933. This variety has been shown by Kosswig (1935) to be a Mendelian recessive to the normal dark form. The histological details of the color pattern of the two varieties are described by Goodrich and Smith (1937).

COLOR REACTIONS

The adaptations of the paradise fish to various colored backgrounds have been studied by methods used by previous investigators (*cf.* Mast, 1916; Connolly, 1925, and bibliography by Parker, 1930). The fish were subjected to tests in small aquaria with white, black, red, yellow, and blue paper jackets and uniform illumination from above. Macroscopic observations on the normal dark type showed typical color adaptations to each environment. The color responses to the red and the yellow backgrounds were relatively similar but yet seemed to be definitely distinguishable. The albino fish, in which melanophores are entirely lacking (Kosswig, 1935; Goodrich and Smith, 1937), showed differing responses against black, white, red and yellow backgrounds. The greatest contrast, however, was between the very pale appearance, when placed against the white background, and the

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various slightly differing combinations of yellow, orange and red given in the reactions to the black, yellow and red backgrounds.

The results of microscopic examinations of the changes in the chromatophores are summarized in Table I. Twenty normal dark fish were available but only four albinos. Every one, however, of these albinos was tested against each background. It will be noted that in the normal dark fish the black, yellow and red cells react essentially alike to environments of black or white or red by showing the dispersed pigment in the black and red environments and concentrated pigment in the white environment. On the yellow background, however, the pigment in the black cells is concentrated, while that in the yellow and red tends to be dispersed. This gives the fish a light yellow color. The reverse situation occurs in blue surroundings, where the yellow and red cells show concentrated pigment and the black cells dispersed pigment. The reactions of the melanophores are more rapid than those of the other two types. The erythrophores behaved in general similarly to the xanthophores, except that they were slightly slower and less extensive in their reactions.

TABLE I

Chromatophore behavior in the paradise fish

D = dispersed pigment; *C* = concentrated pigment; *I* = intermediate state.

	Dark variety			Albino variety		
	Mel.	Xan.	Ery.	Mel.	Xan.	Ery.
Black	*D*	*D*	*D* and *C*	—	*D*	*D*
White	*C*	*C*	*C*	—	*I*	*C* and *I*
Red	*D*	*D*	*D* and *C*	—	*D*	*D*
Yellow	*C*	*D*	*I* and *C*	—	*D*	*D*
Blue	*D*	*C*	*C*	—	*C* and *I*	*C* and *I*

EXPERIMENTS WITH DENERVATED CHROMATOPHORES

After the study of the color changes and their relation to the activities of the chromatophores, attention was next turned to the problem of the physiological control of the chromatophores. The methods used by G. H. Parker (1934*b*) were adopted.[2] The nerves supplying a part of the caudal fin are severed by making a cut across one of the fin rays. This operation leaves a small area between the cut and the edge of the fin without central nervous control. The normal dark fish lay quietly in the dish when the incision was made, but the albino required an anaesthetic, for which purpose ether was used. The experiments, except as otherwise noted, were carried out at room temperature, which remained relatively uniform, the water temperature averaging 23.5° C.

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[2] Kamada (1937), whose paper has just come to hand, has also induced caudal bands in *Macropodus opercularis*.

Immediately upon sectioning the nerve of a dark-colored, or normal fish the melanophores in the area between the cut and the outer edge of the caudal fin began to disperse their pigment. A clearly-defined dark band formed within one minute and it extended laterally to include half of the area between the cut ray and the two adjoining rays. When the pigment of the melanophores is dispersed, it is more difficult to observe other types of pigment cells. Nevertheless, it could be seen that the pigment in the xanthophores was also dispersed, but the reaction was somewhat slower than that of the melanophores. Three to five minutes was required for a degree of dispersion comparable to that shown in one minute by melanophores. The erythrophores lagged slightly behind the xanthophores in dispersing their pigment. These observations were confirmed later on albinos, where no melanophores obscure other types of cells.

When fish with these dark caudal bands were placed in white porcelain dishes illuminated from above, the dark bands faded in about ten hours, as the denervated melanophores gradually assumed the punctate condition. The reverse reaction, of denervated melanophores assuming the dispersed phase after concentration, is much more rapid, taking place in two to three hours.

As noted above, the xanthophores and erythrophores can be easily observed in the albino. The sectioning of a nerve was followed in one or two minutes by a detectable dispersion of pigment in these cells, which, however, take several minutes to complete the reaction. This band, although it contains no melanophores, is nevertheless striking because the rest of the fin with punctate xanthophores and erythrophores is very light. Figure 1 shows the edge of such a caudal band in the albino fish. Although these albino fish remained under the same conditions as the normal ones, there was a marked difference in that the caudal bands faded in about four hours, or less than half the time required by those in the dark-colored fish.

Certain other experiments devised by G. H. Parker (1934*c*), which have special bearing on the neurohumoral hypothesis, were tried with especial reference to the reactions of the erythrophores. The material was limited, but the results seem to be clear. Dark fish were operated on to produce a caudal band in each and then placed in white dishes until the bands were completely faded. Other cuts were then made producing two fresh dark bands, one on either side of the distal half of the faded band. The chromatophores in the proximal half of the faded band remained concentrated, as were those in other parts of the tail (the new dark bands excepted). In the distal half, however, the chromatophores became dispersed, corresponding to the condition of their immediate neighbors in the fresh dark bands. The dispersal occurs first and to the greatest extent at the sides of the faded band. It will be recognized that these observations parallel those made by G. H. Parker on *Fundulus* and so support the theory of neurohumors as he has applied it to explain the reactions of chromatophores in fish. The humor is thought to spread from the new dark band, where it has been recently liberated, to the faded band and to stimulate there the dispersion of pigment in the melanophores.

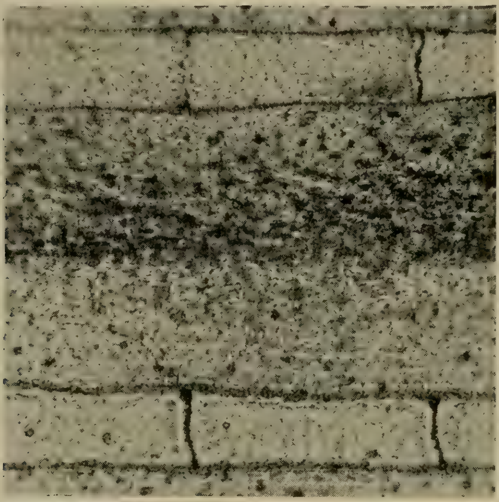

FIG. 1. Photomicrograph of edge of caudal band in albino fish. The pigment cells are erythrophores. 95 ×.

The same experiment was tried on albinos to test the occurrence of a similar dispersing neurohumor governing the erythrophores. A definite expansion was obtained in the distal half of the flanked bands in two albinos in which both lateral bands were successfully induced. It did not occur in two cases where only one flanking band was produced.

The reactions of the denervated melanophores differ from those of the xanthophores and erythrophores. This was shown by observations made when normal dark paradise fish with faded caudal bands were transferred from a white dish to a yellow environment. The melanophores remained concentrated, but the xanthophores and erythrophores became gradually dispersed. Each type of chromatophore assumed the same condition of pigment distribution as the corresponding type of innervated pigment cells in the surrounding area.

TEMPERATURE EXPERIMENTS

As a further test of the neurohumoral hypothesis, an experiment was carried out to determine the effect of temperature on the rate of disappearance of bands produced by cutting nerves in the caudal fins of normal fish. If the fading of such bands is indeed due to the dispersion of an oil-soluble substance from cell to cell, as Dr. Parker's theory indicates, it might be expected that the dispersion should occur more rapidly at a higher temperature than at a lower one, in accordance with laws of diffusion. Accordingly, fish were operated on as previously described for the formation of dark caudal bands and isolated in white porcelain dishes illuminated from above. Two determinations were made with each fish, one at a temperature of 20° C. and the other at about ten degrees higher. The bands were considered faded when they could no longer be distinguished by the unaided eye when the fish were observed in a paraffin-lined Petri dish with a white surface in the background. The following table summarizes the data obtained:

TABLE II

Effect of temperature on rate of caudal band disappearance in normal fish

Observations 1–4: Low = 20° C., high = 29° C. Observation 5: Low = 23° C., high = 27° C. Figures are elapsed hours after operation when bands became so faint as to be practically indistinguishable.

	Low	High
1	18 hrs.	8 hrs.
2	10	5
3	10	5
4	10	5
5	7.75	3

The results of this experiment are what would be expected on the basis of the neurohumoral hypothesis; i.e., that the dark bands disappear more rapidly at a higher temperature than at a lower one. Furthermore, it appears that with a rise in temperature of approximately ten degrees, the time required for disappearance is reduced very nearly one half.

DISCUSSION

The action and control of chromatophores in the paradise fish are found to be similar to that reported for certain other species. The rate of reaction of these processes, however, is strikingly different.

This disparity shows particularly in the rate of disappearance of dark bands produced by denervating chromatophores in the caudal fin. Caudal bands in *Fundulus* (Parker, 1934*a*) fade in twenty-two to ninety-six hours, while those in the catfish (Parker, 1934*b*) fade only after two or three days and may last as much as seven days. In contrast to these conditions, bands in the normal paradise fish fade in approximately ten hours, and in the albino, in about four hours. The activity of the concentrating neurohumor, then, is more rapid than in the fish previously reported.

This greater speed of action applies also to the dispersing neurohumor. Parker (1934*a*) reported that in *Fundulus* light bands, produced by transferring fish with faded caudal bands from white surroundings to black, fade in seventeen to twenty-seven hours. In the paradise fish, however, similar bands fade in only two and a half hours.

That caudal bands in the albino should disappear in approximately half the time taken by fading bands in the normal fish is a puzzling problem. Conceivably the neurohumor (or humors) produced in regulating the xanthophores and erythrophores might be more soluble than that for controlling melanophores and, consequently, become dispersed more readily than the latter. At any rate, a difference in chemical or physical properties of the two substances seems to be indicated.

The independence of action of melanophores and xanthophores is similar to that described by Abramowitz (1936) for *Fundulus majalis.* In the paradise fish, however, the erythrophores have also been studied and their reactions are found to be similar to but a trifle slower than those of the xanthophores. This gives additional evidence of the possible close relationship between xanthophores and erythrophores as suggested by Goodrich and Smith (1937).

The results obtained which show that an increase of temperature brings about an increase of rate of disappearance of the dark caudal bands may have bearing on the theory of neurohumoralism. It supports the concept that the effect is produced by a diffusing chemical substance.

SUMMARY

1. The normal paradise fish adapts itself by appropriate color changes to environments of black, white, red, yellow, and blue.
2. Analogous but less adaptive changes occur in the albino.
3. The reactions of melanophores, xanthophores and erythrophores which produce these color changes are described.
4. Dark caudal bands, formed by cutting chromatophore nerves in the caudal fin of normal paradise fish fade in approximately ten hours. Similar bands in the albino fish fade in about four hours.

5. The evidence indicates the presence of independent dispersing neurohumors for melanophores and for erythrophores.

6. The rate of caudal band disappearance is directly proportional to the temperature, and a rise of about ten degrees in temperature very nearly doubles this rate.

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AUTOSOMAL LETHALS IN WILD POPULATIONS OF DROSOPHILA PSEUDOÖBSCURA

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INTRODUCTION

It has been recognized for some years, following the work of Muller, that lethals are especially convenient material for the study of mutation rates. This is because they occur with a frequency that is great enough to be measured, and because their occurrence can be detected by a technique that is independent of the personal equation of the observer. These same two advantages apply to the use of lethals in the study of the constitution of wild populations. Two other advantages are also to be noted in this field. Owing to the extensive studies on the mutation rates of lethals, there is available a large body of data on the frequency of the occurrence of new lethals under a variety of conditions. On the other hand, the rate of elimination of lethals from a population furnishes the minimum possible difficulty of quantitative estimation. It is, therefore, not surprising that there is a rapidly increasing literature in the field—to which the present paper belongs.

MATERIAL AND METHODS

A large series of wild strains of *Drosophila pseudoöbscura* have been studied in this laboratory (see, for example, Dobzhansky, 1935; Dobzhansky and Boche, 1933; Sturtevant and Dobzhansky, 1936). Tests have been carried out on a series of these, to determine the frequency with which autosomal lethals occur. Most of the work has been with the third chromosome, though a few tests on the second will be described below.

Wild males, or a single son from each of a series of wild females (i.e., females already fertilized when trapped), are mated individually to females carrying the recessive gene for orange eyes. From each such mating a single son is mated to females of the multiple stock orange Scute (dominant, bristle reduction) purple (recessive, eye color). All the Sc, not-or, offspring of this mating carry one particular third chromosome from the wild specimen; when they are mated together $\left(\frac{+}{\text{or Sc pr}} \times \frac{+}{\text{or Sc pr}}\right)$ the not-Sc offspring are homozygous for one of the two third chromosomes of the original wild male. If this chromosome carries a lethal, the not-Sc class is absent.

The difficulty with the method is that, as just outlined, it neglects the possibility of crossing over. This possibility exists only in the females used in the final test generation, since everywhere else the tested chromosome is kept in males, in which crossing over is absent (or at most negligible in frequency). Even in the females in question, it happens that in the majority of cases crossing over is not a serious source of error. As reported by Sturtevant and Dobzhansky (1936), there is a wide variety of sequences in this chromosome. The or Sc pr stock has the Standard sequence; and females heterozygous for Standard and any other sequence give few crossovers. The presence of the three mutant genes in the experiment makes it possible to distinguish between Standard, on the one hand, and all the remaining sequences, on the other hand, in the tested third chromosome. In case the tested chromosome does not have the Standard sequence (and the majority of them do not have it) the presence of a lethal is at once apparent from the absence or extreme rarity of wild-type flies in the test generation. Most of the sequences do give occasional crossovers in one region or another when tested against Standard; for this reason special tests are required to distinguish between semi-lethals and lethals that cross over with Sc. These have not, in general, been carried out in the present experiments; but it is clear that very few semi-lethals are present, since in most cases the wild-type class is either wholly absent or approximately half as numerous as the heterozygous Scute class. This results from the fact that few of the lethals happened to lie in the sections that give crossovers with Scute.

If the test chromosome has the Standard sequence, lethals can still be detected, though it is necessary to make more extensive counts to be certain no lethal is present. This is due to the fact that Sc lies near the middle of the chromosome (about 30 units from the left end, 40 from the right), so that any lethal shows appreciable linkage with it. Here, however, the distinction between lethals remote from Sc, and low-viability genes close to it, requires special tests. The test, applied in most cases, has been to use a chromosome carrying the dominant Emarginate (eye shape) and the Arrowhead sequence. From the mating $\frac{\text{Em}}{+} \times \frac{\text{Em}}{+}$, results comparable to the Sc test outlined above are obtained.

In the case of the second chromosome, the test procedure is similar. Wild males or single sons of wild females are mated to glass (recessive, eye structure) females, and a single male from each such mating is mated to Bare (dominant, bristle form) glass females. The resulting Bare offspring $\left(\frac{\text{Ba gl}}{+}\right)$ are mated together, and counts are made of their offspring. This test is less efficient than that used for the third chromosome, since there is more crossing over. No inversions are present in the material tested, and Bare is about 60 units from the left end of the chromosome, 40 from the right. Lethals even at this distance do cause a disturbance of the ratios; and all suspected lethals were verified by making a series of matings of Ba × Ba from sibs. In every case these tests agreed in showing a lethal to be present.

TABLE I

Frequency of lethals

	Chromosomes tested	Lethals	
		Number	Percentage
Chromosome II			
1936	21	4	19.0
Old stocks	16	3	18.8
Chromosome III			
1935	67	13	19.4
1936	120	23	19.2
Old stocks	29	7	24.2

However, there is no test for distinguishing lethals and semi-lethals; and the technique is more laborious than that used for the third chromosome. For these reasons relatively few tests were made on the second chromosome.

The mutant strains available did not permit reasonably efficient tests for lethals in chromosomes IV or V, and none was attempted. In the case of the X chromosome no detailed tests were made, but observations on the sex ratio from wild females and their daughters indicated that no lethals were present in any of the wild strains studied.

It should be pointed out that what is being studied here is lethal-bearing chromosomes, rather than lethals. That is to say, if a chromosome carries more than one lethal the technique used does not make the fact evident. With frequencies of lethals as high as those found it seems likely that such duplications of lethals in a single chromosome do in fact occur. The net result will be that the frequencies recorded are too low. Further complications arise in connection with the tests for identity of lethals, but since few cases of identity were found this does not seem a serious source of error.

The results obtained are shown in summary form in Table I.

The material studied in 1935 came largely from the Rocky Mountains and from the Mexican plateau; that in 1936 largely from southern California. The "old stocks" were, many of them, from the Pacific Northwest. These latter had been kept in the laboratory for at least a year before the lethal tests were begun. The striking thing about the table is that, in spite of these differences, there was little variation in the frequencies found for a given chromosome or even as between the second and third chromosomes. Table II, giving the data for the most-studied individual localities (included in Table I) shows a similar relative constancy of the frequency of lethals. All these results are from Race A; 5 Race B third chromosomes (all from the Olympic Peninsula of Washington) were tested, and one lethal was found, giving a frequency of 20 per cent.

TABLE II

	Chromosomes tested	Lethals	
		Number	Percentage
Chromosome III			
San Gabriel Canyon, Azusa, Calif.	27	8	29.6
Julian, Calif.	21	3	14.3
San Gorgonio Mt., Calif.	17	3	17.6
Santa Cruz Isd., Calif.	16	3	18.8

As reported by Sturtevant and Dobzhansky (1936), the third chromosomes of this species exist in a wide variety of sequences. This phenomenon does not appear to be related to the occurrence of lethals. Lethals have been found in the Standard, Arrowhead, Pikes Peak, Santa Cruz, Chiricahua, Cuernavaca, and Klamath sequences. Their frequency seems to be essentially the same in regions such as southern California or southern Mexico, where several different sequences exist in fairly large numbers within each population, and in the area near the common corner of Utah, Colorado, Arizona, and New Mexico, where only the Arrowhead sequence is found. Fifteen third chromosomes tested from this pure Arrowhead area gave 3 lethals—exactly 20 per cent. The data on Chromosome II also indicate that diversity of sequences is not important, since all the strains from the regions concerned in these tests had the Standard Race A sequence in that chromosome.

TESTS FOR ALLELOMORPHISM

If two lethals, different in origin, are kept in stocks of the form $\frac{\text{lethal}}{\text{or Sc pr}}$, it is easy to test their identity by crossing the two strains. If two distinct lethals are concerned, wild-type offspring will appear in numbers approximately equal to half the size of the heterozygous Scute class; if the two lethals are identical, no wild-type offspring will be produced except as a result of crossing over in the female used, and this will be infrequent if the lethal-bearing chromosome does not have the Standard sequence. Numerous tests of this kind have been carried out.

Fifteen of the 1935 and "old stock" lethals were tested in all possible combinations; the result showed 14 lethals to be present. One from Metaline Falls, Washington (1934) was identical with one from Florence, Texas (1935). Thirteen of the 1936 lethals were tested against each other, and were found to include 12 different ones. One of three tested from Julian was found to be identical with one of eight from San Gabriel Canyon. The Metaline 1934 lethal was tested against the 12 different 1936 lethals, and was found to be allelomorphic to one from Barton Flats, on the slopes of Mt. San Gorgonio, Calif. The San Gabriel-Julian duplicate was different from all the seven surviving members of the 1935 series with which it was tested. These tests, with a few miscellaneous others, total 225 crosses between separately found lethals, with 3 cases of allelomorphism. That is, an average of about $\frac{3}{225} = 1.3$ per cent of the lethals found may be expected to be allelomorphic to any given one selected at random. Or, since the total frequency of lethals in this chromosome in wild stocks is just under 20 per cent, the average frequency of a given lethal may be estimated at about one-fourth of 1 per cent.

It may be observed that no cases of recurrence have been found within a locality. That is to say, the same lethal has not been recovered from any two specimens collected in the same place. As shown above, one lethal was found both at Julian and at San Gabriel Canyon, in southern California, in 1936. While the two localities are only about

100 miles apart, the populations of their respective mountain ranges (San Jacinto and Sierra Madre) appear to be distinct in that the former contains the Santa Cruz sequence while in the latter Tree Line occurs (along with Standard, Arrowhead, and Chiricahua, common to both regions—see Dobzhansky and Sturtevant MS in press—*Genetics*). The other recurrent lethal was found in stocks from Metaline Falls, Washington (1934), Florence, Texas (1935), and Mt. San Gorgonio, Calif. (1936). It seems clear in the latter case, and probable in the former, that we are dealing with recurrent mutations rather than with the persistence of a lethal gene in heterozygous form.

Rate of Origin of New Lethals

A preliminary lethal-accumulation experiment has been carried out. The technique used is not essentially new, and the results are not yet extensive. It seems sufficient at present, therefore, to record that 2 new lethals occurred in a total of 120 chromosome-generations, giving a frequency of 1.7 per 100—with a large probable error.

Properties of Non-lethal Chromosomes

The frequency of lethals found in the tested wild stocks is unexpectedly high. A possible explanation of this result seemed to be that the species is in a condition approximating that of balanced lethals. That is to say, that most or all its third chromosomes are so constituted that flies homozygous for any one of them cannot compete with heterozygotes. Such an assumption amounts to supposing that the phenomenon of heterosis is well developed within a single pair of chromosomes. Under these conditions it might result that the rate of elimination of a lethal would be greatly decreased; since lethals are eliminated only when homozygous, and the assumption is that homozygotes play little part in the perpetuation of the species, so that their properties are unimportant.

The simplest way to test this hypothesis is to study the properties of individuals homozygous for a series of non-lethal chromosomes, from the same wild populations as those that contained lethals, in comparison with individuals heterozygous for two such chromosomes. There is another question that can conveniently be investigated at the same time—are the lethals completely recessive? This latter question is of importance in any calculation of the probable rate of elimination of lethals from wild populations.

Only one characteristic of the flies concerned has been investigated —namely their viability. It is clear that fertility, length of life, resistance to unfavorable conditions, and other properties, would all be of importance in determining the frequencies found in natural populations; but the labor involved in studying a series of combinations for such a variety of characteristics has seemed prohibitive. It also seems probable, *a priori*, that there will be, in general, a rough correlation between all these properties, such that data on one of them will give a satisfactory picture of the general situation.

The technique used was the same as that used in the detection and testing for identity of lethals—in fact many of the cultures served both purposes. For the study of lethals partial counts were adequate, and were used in many cases. However, if complete counts are made on a culture there results a measure of the viability of the wild-type chromosome concerned. From the mating together of $\frac{+}{\text{or Sc pr}}$, in the absence of crossing over, three types are expected in the ratio 1 : 2 : 1. The homozygous or Sc pr is usually present in rather small numbers, and has been neglected in making comparisons, since the numbers seem to be very much influenced by minor environmental differences and are often so low as to be inconveniently sensitive to the error of random sampling. The index of viability actually used is the number of wild-type ÷ the number of heterozygous Scute × 100—i.e., the percentage that the wild type is of the Scute. Evidently, with equal viability of all classes the index will be 50.

Several objections may be raised to this method. The most serious is that each of the classes with which we are concerned is compared, not with a common standard type, but with a $\frac{+}{\text{or Sc pr}}$ class that varies in constitution from one test to another. In the case of a test of a homozygote the comparison concerns a heterozygote of that same chromosome; in the test of a heterozygote the $\frac{+}{\text{or Sc pr}}$ class is made up of heterozygotes for each of the two + chromosomes, in approximately equal numbers. In a few cases tests were repeated, and the result shows a definite, though far from complete, correlation between successive tests of a given combination. The use of this technique can, therefore, not be considered as giving more than an indication of the situation, and accordingly it does not seem desirable to present the results obtained in detail.

In general, the homozygotes for non-lethal third chromosomes from wild stocks are viable and fertile in both sexes, with the result that homozygous stocks are easily established. The test outlined above indicates that some homozygotes are definitely below par, others apparently at no disadvantage. The average viability index of 21 tested chromosomes was 45.91, the minimum 19.4 (weighted average of three tests—16.3, 21.6, 33.3).

The tests of heterozygotes for two-third chromosomes from wild strains were made chiefly with lethal-bearing chromosomes. Twenty-five different combinations of lethal × lethal gave an average index of 53.0—i.e., more viable than the separate lethals heterozygous for or Sc pr. The lowest index among the 25 was 38.3.

The most probable conclusions are that

(1) lethals are wholly recessive;

(2) homozygotes for non-lethal chromosomes are slightly less viable, on the average, than heterozygotes;

(3) the viability of homozygotes is quite variable, some being definitely low, others apparently as high as the heterozygotes.

Comparison with Results from D. melanogaster

The frequency of lethals in wild populations of *D. melanogaster* has been studied by several authors. The most extensive work is that of Dubinin and co-workers (1934, 1936) on material from the Caucasus. These studies were carried out on a large scale. Altogether 4819 second chromosomes are recorded, with 470 (= 9.8 per cent) lethals. The frequencies range from 0 (in 92 tested, from Delizhan) to 16.1 per cent (161 tested, from Ordzhonikidze). From one locality, Gelendzhik, tests were made in three successive years, giving 7.98 per cent (877 tested), 12.86 per cent (616 tested), and 8.78 per cent (797 tested). Tests were also carried out for identity, but only within regions. The average value here was 2.2 per cent—i.e., an average of 2.2 per cent of the lethals found in one year at one locality may be expected to be allelomorphic to any one lethal found in that year at that locality. The average frequency for a given single lethal is 0.22 per cent (.022 × .098). There are no tests for identity of lethals from different localities, but those found at Gelendzhik in successive years were so tested, with the result that "among the 33 lethals of 1933 and the 55 lethals of 1934, nine lethals were common to both years." The conclusion is drawn that this represents survival of these lethals. The results reported in the present paper throw some doubt on this conclusion, for a comparable frequency of identity was found in *pseudoöbscura* for all years and all localities. One may surmise that, in *melanogaster* also, any two series of lethals would show a fairly large proportion of common members—i.e., that recurrent mutations are relatively frequent.

The absolute frequency of lethals found in the Caucasian *melanogaster* populations is only about half that in the American *pseudoobscura*, though the chromosome concerned (II) is roughly twice as long as the III of *pseudoöbscura* and includes the same material plus that of the *pseudoöbscura* IV (Donald, 1936; Sturtevant and Tan, 1937). The preliminary experiment described above suggests a higher lethal mutation rate in *pseudoöbscura;* but until more data are available on this point it is scarcely possible to decide on the relative rates of elimination of lethals in the two species.

One difficulty in evaluating the data of Dubinin and his co-workers may be pointed out. The results are recorded by localities and years; but we are nowhere given more specific data. How large an area is included in a single "locality"? Over how long a period were specimens taken in one year? What kinds of places were collected—woods, grocery stores, garbage dumps, fruit orchards? These questions are of importance in judging the size of the populations sampled and the probable degree of relationship of the tested individuals.

SUMMARY

1. Approximately 20 per cent of the third chromosomes found in wild populations carry lethals.

2. Less extensive data indicate a similar frequency for the second chromosome.

3. On the average, about 1.3 per cent of the lethals found may be expected to be allelomorphic to any given one.

4. The average frequency for any one lethal is about one-fourth of one per cent of the chromosomes of wild strains.

5. The lethals, so far as studied, are completely recessive.

6. Study of flies homozygous for non-lethal third chromosomes shows a considerable variation in their viability. Some are at a definite disadvantage, others apparently not. On the average they are not quite as viable as flies carrying two different third chromosomes.

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THE EFFECT OF SALINITY UPON THE GROWTH OF EGGS OF FUCUS FURCATUS[1]

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INTRODUCTION

The effect of salinity upon fertilization and early development in 3 species of *Fucus* (*F. serratus*, *F. vesiculosus*, and *F. spiralis*) was studied by Kniep (1907) on the Norwegian coast. He investigated especially the tolerance for dilution of the sea water and found it to differ considerably in the different species. Kniep was able to correlate the tolerance with the distribution of the species (and, by inference, of additional species of *Fucus* and other related marine algæ as well) especially in the Baltic Sea and in near-by regions in which brackish water communicates with the ocean, thus establishing gradients of salinity.

The present investigation is an attempt to measure the effect of increased and decreased salinity upon the percentage germination and the growth rate of the fertilized eggs of *Fucus furcatus f. luxurians* throughout the range of salinity in which germination and growth take place, and to do so under strictly controlled conditions. The eggs were transferred abruptly into sea water of altered salinity, and therefore, any effect which a prolonged gradual adaptation might have on the tolerance is eliminated from the measurements. The data are useful in conjunction with other experimental investigations of the development of this egg in which the salinity of the medium varies.

MATERIAL AND METHOD

Fruiting tips of this hermaphroditic *Fucus* were collected at Moss Beach, California in May, June and July, 1937. The material was cared for in the manner described in an earlier paper (Whitaker, 1936). Sea water was collected at the same place and was filtered twice upon arriving at the laboratory. In each experiment, eggs from the same batch were reared in this sea water and also in samples of the same sea water with artificially altered salinity. Salinity was decreased by adding triple glass-distilled water. It was increased by boiling under

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[1] This work has been supported in part by funds granted by the Rockefeller Foundation.

vacuum so that the temperature did not rise enough to cause precipitation of salt. By this method the volume of sea water was reduced to half without precipitation. The measure of salinity which has been used is the total salt content per unit volume expressed as a percentage of the total salt content per unit volume of normal sea water. In all cases hydrometer readings were made which confirmed the salinity as originally determined volumetrically. The specific gravity of the normal sea water used was 1.027.

The pH of the samples of normal sea water, as determined with a glass electrode, ranged from 7.9 to 8.2. The pH tended to rise slightly when the salinity was artificially increased, and to fall when it was decreased. Such shifts were usually not great, being of the order of a few tenths of a pH unit or less in most experiments. In the most extreme cases the shift amounted to 0.5 units. The maximum pH range of the media in the experiments upon which Fig. 1 is based was 7.7 to 8.5. In some of the experiments, all of which gave essentially similar results, the range was considerably less. This pH range is much narrower than the limits of normal development for this species of *Fucus* (Whitaker, 1937), and it is small enough so that it appears safe to conclude that it is a minor factor in the present results.

Since *Fucus furcatus* is hermaphroditic, and sheds male and female capsules together at the same time, it is not feasible to inseminate a population of eggs at any precise moment. The male capsules dissolve in the sea water first, however, so that fertilization takes place when the egg capsules dissolve and liberate the individual eggs into the sperm bearing sea water. By selecting eggs only from capsules which break down during a limited period, it is possible, in effect, to confine fertilization to this period. In these experiments eggs were used which were fertilized during a 10-minute period.

In order, for the present purpose, to rule out the effects of altered salinity upon fertilization and entrance of the sperm, eggs were fertilized in normal sea water in all experiments. Twenty minutes after the end of the fertilization period samples were transferred with a negligible amount of normal sea water into the media of altered salinity. Eggs had thus been fertilized 20–30 minutes when they were removed from normal sea water. They were thinly seeded in covered Petri dishes which were stored in a moist chamber in a dark, humid, constant temperature room at 15° C. Fertilization and all subsequent development took place in this constant temperature room. The eggs were observed only with red light until the end of the experiment.

RESULTS

The fertilized *Fucus* eggs develop in a relatively great range of salinity. As the limits of tolerance are approached, all eggs in a population do not have the same end point, and the results are represented graphically by the blocks in Fig. 1 to show the percent of the eggs in the populations which form rhizoids. Each block in the figure represents the average of the results of from 4 to 10 experiments, each involving counts on 300 or 400 eggs. The counts were made two days after fertilization.

The blocks in Fig. 1 show that very high percentages of the eggs develop in sea water ranging in salinity from 50 or 60 to 150 per cent of that of normal sea water. Beyond these limits the percentage drops off rapidly, although some eggs develop rhizoids when the salinity is as extreme as 30 per cent or 180 per cent. Beyond these limits no rhizoids are formed. In the more dilute sea water (10 per cent and 20 per cent) eggs burst and cytolyze at once, and many of the eggs which fail to form rhizoids in 30 per cent or 40 per cent sea water also cytolyze. In the concentrated sea water, on the other hand, most of the eggs which fail to form rhizoids do not cytolyze, at least in the course of several days. Eggs which have remained for two days in sea water of twice the normal salinity form rhizoids after being returned

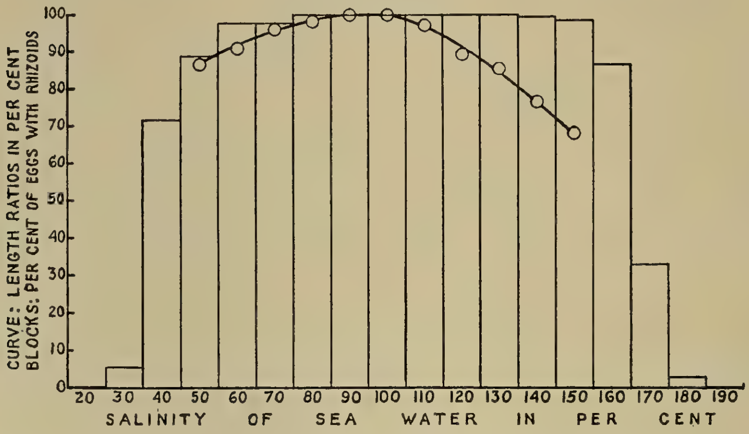

FIG. 1. Blocks: The percentages of eggs which formed rhizoids in normal sea water, in diluted sea water and in concentrated sea water, as observed after two days.

Curve: The average over all length of embryos at 92–96 hours, reared in sea water of the salinity indicated, expressed as a percentage of the average length of control embryos reared in normal sea water.

In all cases the eggs were fertilized and reared in the dark at 15° C. The specific gravity of the normal (100 per cent) sea water was 1.027 (see text).

to normal sea water. The suppression at high salinity thus bears some resemblance to anesthesia.

The *Fucus* egg is spherical until a bulge forms on one side, about 16 hours after fertilization (Whitaker, 1936). This bulge extends in filamentous fashion by elongation and cell divisions until its length is many times the diameter of an egg. Ultimately the rhizoid of the new plant forms from this filamentous structure, while the remainder of the embryo gives rise to the thallus. The rate at which early development takes place, as indicated by the rate of extension of the rhizoid filament, is a function of the salinity of the medium. In both extremes of the range of salinity, in which many or most of the eggs form no rhizoids at all, the rate of extension is greatly retarded in those eggs which do form rhizoids. Within the ranges of salinity (50 or 60 to 150 per cent, see blocks, Fig. 1) in which practically all of the eggs form essentially normal rhizoids, the salinity affects the rate of extension as shown by the curve in Fig. 1.

The curve in Fig. 1 shows the average full length of the embryos reared in media of the salinities indicated, expressed as a percentage of the average full length of the control embryos from the same batches of eggs reared in normal sea water. The lengths were measured with an occular micrometer 92–96 hours after fertilization. Each point was obtained by averaging the results of 4 to 10 experiments, each involving counts of 50 or 100 eggs, except that the point at 90 per cent salinity is based on only 3 experiments. The results of the separate experiments are very similar.

It is seen from the curve in Fig. 1 that the embryos grow as rapidly in 90 per cent as in 100 per cent sea water. The average absolute length of the embryos in these optimum salinities was 303 microns at 92–96 hours. Since the embryos were growing in the dark in the absence of photosynthesis, no nutrients were available to the eggs except those stored in the unfertilized eggs. It is therefore doubtful if much new protoplasm was synthesized, although internal conversion of stored foodstuffs may have supported some protoplasmic synthesis. The growth or extension observed is presumably largely a developmental elaboration, i.e., an extreme change of form.

SUMMARY

1. Fertilized eggs of *Fucus furcatus f. luxurians* have been reared in the dark at 15° C. in diluted and concentrated sea water.

2. When the salinity of the medium is between 60 per cent and 150 per cent that of normal sea water (sp. gr. 1.027), practically all of the eggs in a population form rhizoids and develop. The rate of elongation

of the embryos, as measured at 4 days, is the same when the salinity is 90 per cent or 100 per cent. When the salinity is greater or less, the growth rate is retarded as shown in the curve in Fig. 1.

3. As the salinity is reduced below 60 per cent or is increased above 150 per cent, the percentage of eggs which form rhizoids declines rapidly as shown in the blocks in Fig. 1. In 10 per cent and 20 per cent sea water the eggs burst and cytolyze. In concentrated sea water which inhibits development the eggs do not cytolyze and the developmental inhibition may be reversible.

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STIMULATION AND NUCLEAR BREAKDOWN IN THE NEREIS EGG[1]

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When a protoplasmic system is exposed to a so-called stimulating agent, a series of changes occurs which results eventually in activity of the protoplasm. Various opinions have been expressed as to the physico-chemical basis of stimulation. In our laboratory for several years we have been interested in proving that stimulating agents all cause a release of calcium from a calcium proteinate gel in the cell cortex. This calcium is then supposed to initiate a protoplasmic clotting similar in some ways to blood clotting. The evidence on which this theory is based has been summarized in a recent book (Heilbrunn, 1937, Chapter 37), and the details of the theory are also given there.

Although in previous studies various types of animal and plant material have been used, and the theory has thus been applied to cells very different morphologically, as yet no attempt has been made to determine what effect stimulation may have on the cell nucleus. Certainly in a dividing cell, the nucleus plays an all-important rôle. It is of interest, therefore, to inquire what effect stimulation may have in those cases in which the primary result of stimulation is cell division. As is well known, the egg cells of many marine invertebrates are sensitive to a wide variety of stimulating agents, and following stimulation, division is usually initiated.

Typically, during mitosis the nuclear membrane breaks down. The mechanism of this breakdown has scarcely been investigated experimentally, although an understanding of the causes of such nuclear breakdown would be an important link in any complete theory of cell division. In the case of many marine invertebrates, the egg is released into the sea water in an immature condition, and it contains a large nucleus or germinal vesicle. Sometimes contact with sea water is in itself sufficient to cause a breakdown of the germinal vesicle and an initiation of the maturation or polar body divisions. This is true of the starfish egg. In other instances, the egg remains immature and retains its large germinal vesicle until insemination. Then the entrance of sperm into the egg, or its contact with the egg cortex, provides the stimulus for nuclear breakdown and maturation divisions. The egg of the annelid worm *Nereis limbata* has a large germinal vesicle when it is shed into sea water, and this is broken down after insemination. The *Nereis* egg has been widely used both in studies of normal fertilization (Lillie, 1911), as well as in studies of artificial parthenogenesis (Just, 1915; Heilbrunn, 1925). It is readily obtainable at Woods Hole, and is an easy object to study. Following activation, the breakdown of the germinal vesicle can readily be detected in the living egg, especially if the egg is somewhat compressed between slide and coverglass.

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Some years ago, Just showed that the *Nereis egg* could be artificially activated by heat (Just, 1915; see also Heilbrunn, 1925). Later, Just (1933) found that ultra-violet radiation could also cause activation. In general, it is believed by radiologists that Roentgen rays can produce the same effect as ultra-violet radiation. Accordingly an attempt was made to discover if activation could be produced by Roentgen rays. It was found that in order to obtain results, extremely high dosage was necessary. In three experiments in which eggs were exposed to 7600 r units per minute for 75 minutes, the percentage of eggs activated was found to be 74, 52, and 5. Three other experiments gave negative results. The activation was not the result of heat, for the dishes containing the eggs were packed in ice to keep them from becoming overheated.

The *Nereis* egg may be activated by chemical agents as well as by physical means. It was found that isotonic solutions of sodium chloride cause a high percentage of germinal vesicle breakdown. Similar effects may also be obtained with isotonic solutions of potassium chloride. Doubtless various other chemical agents are also effective, but no attempt was made to list all the effective reagents. However, it may be noted that a dilute ether solution (3 per cent in sea water) is somewhat effective in causing germinal vesicle breakdown. Other fat solvents doubtless act in similar fashion.

The literature on artificial parthenogenesis contains many papers in which activation of marine eggs has been found to follow various types of treatment. Our concern has not been to prepare an exhaustive list of the effective agents for the *Nereis* egg. We have attempted to discover the nature of the effect of several physical and chemical activating agents, and we have assumed that if we can throw light on the true mechanism of activation in two or three cases, such information may perhaps be applied to all types of activation.

In our experiments we have studied the action of ultra-violet radiation and of isotonic salt solutions, and we have tried to discover whether the calcium release theory of stimulation will hold for these cases. Our experimental procedure was very simple. First we determined the percentage of germinal vesicle breakdown following exposure to the stimulating agent. Then we immersed eggs in citrate solutions, to discover if previous exposure to citrate would prevent activation. As is well known to students of blood clotting, citrate solutions tend to prevent the action of calcium. Opinions as to the mechanism of the effect differ. (Compare Hastings, McLean, Eichelberger, Hall and DaCosta, 1934.) Some unpublished work of D. Mazia indicates that when sea-urchin eggs are immersed in citrate solutions, a large percentage of the calcium normally present in the cell cortex is removed. If citrate solutions are able to remove calcium from the cell cortex, or if they prevent release of calcium to the cell interior by some other mechanism, then citrated eggs should be incapable of activation. This was found to be the case.

TABLE I

Nuclear Breakdown following Irradiation

Percentage breakdown after 60 seconds irradiation in sea water	Immersion time in 0.35 M Na citrate	Percentage breakdown after 60 seconds irradiation in Na citrate
	minutes	
100	$3\frac{1}{2}$	0
100	4	7
98	4	7
100	4	2
100	4	6
40	4	5
100	4	2
100	4	37
92	4	11
97	4	5
100	5	0
100	5	5
100	5	0
97	5	19
97	29	0

Two types of experiments were tried. In the first place, eggs were exposed to ultra-violet radiation, both in sea water and in sodium citrate solutions. Ultra-violet radiation was obtained from a Uviarc Laboratory Outfit. This type of lamp is well known and its characteristics have been described in a paper by Buttolph (1930). The lamp was operated at 160 volts and 5 amperes. In our work we were not interested in the energy output of the lamp nor in separating different wave-lengths of radiation. A few preliminary tests soon showed that when *Nereis* eggs were placed in a small quantity of sea water and exposed to the mercury arc at a distance of 26 cm. for 60 seconds, all of the eggs, or at least more than 90 per cent of them, showed activation. Shorter exposures (e.g. 30 seconds) were not quite so successful. On the other hand, following exposures of 3 or 4 minutes, slightly lower percentages of activation were obtained. It is thus clear that under the conditions of our experimentation, a 60-second exposure to the Uviarc lamp was an effective stimulus, and we have reason to believe that other Uviarc lamps act approximately as ours did, unless of course, the lamps have suffered very serious deterioration. In any case, it is a simple matter to determine the exposure necessary to obtain activation, for there is a wide range over which this occurs.

TABLE II

Nuclear Breakdown in Isotonic NaCl

Percentage breakdown in NaCl	Immersion time in 0.35 M Na citrate	Percentage breakdown in NaCl following citrate treatment
	minutes	
99	4	4
96	4	4
99	4	7
93	4	24
35	5	0
86	6	2
88	6	0
69	6	0
89	6	0
100	6	0
100	6	1
100	6	0
95	6	0
94	6	1
99	6	0
100	8	2

Table I shows the percentages of nuclear breakdown following irradiation in sea water, as compared with the percentages obtained following irradiation in citrate solutions. The difference is striking. In sea water typically, practically all the eggs respond by nuclear breakdown. On the other hand in the citrate solution, there is almost no response.

Similarly, when eggs are exposed to isotonic sodium or potassium chloride solutions (0.53 molar), there is a high percentage of response, but if the eggs are first immersed in 0.35 molar sodium citrate and then exposed to the sodium or potassium chloride solutions, the eggs fail to respond. This is shown in Tables II and III. From these tables it will be noted that although a 4-minute immersion in the citrate solution is sufficient to prevent most of the eggs from showing nuclear breakdown, occasionally there is some response following such immersion. Thus in the fourth experiment of Table II, 24 per cent of the eggs were activated after 4 minutes of citrate treatment. If the eggs are allowed to remain in the citrate solution for 6 minutes before being transferred to the sodium chloride or the potassium chloride solution, there is almost no nuclear breakdown. Apparently, the citrate solution must act 5 or 6 minutes if it is to be completely effective in preventing response to stimulation.

TABLE III

Nuclear Breakdown in Isotonic KCl

Percentage breakdown in KCl	Immersion time in 0.35 M Na citrate	Percentage breakdown in KCl following citrate treatment
	minutes	
40	4	18
93	4	10
100	4	Some eggs broken up. Count impossible.
100	4	Some eggs broken up. Count impossible.
52	5	0
93	6	1
28	6	0
11	6	0
81	6	0–2
44	6	0
71	6	0
43	6	0
99	6	0
100	6	0
99	6	0
100	8	4

In these studies of activation, our attention was focussed on the nuclear breakdown, and no attempt was made to study the cortical changes which occurred in the eggs. These changes are interesting, but they were not considered in the present study.

It might well be urged that the effect of the citrate in preventing the stimulating effect of ultra-violet radiation and of sodium and potassium solutions was essentially due to injury. Thus, if eggs are fixed in formalin or some other poison, they presumably would not respond to any type of stimulation. This objection is very easily met. If citrated eggs are returned to sea water, they immediately become sensitive to radiation again. This is clearly shown in Table IV. Eggs exposed to citrate for 6 minutes and then returned to sea water show nearly 100 per cent of germinal vesicle breakdown on irradiation. In this connection, it should be pointed out that even without irradiation, a certain percentage of eggs may show germinal vesicle breakdown after transfer from the citrate solution to sea water. This is an interesting fact and will be considered again later.

As a whole, our results show that both physical and chemical stimulation of the *Nereis* egg, as evidenced by the breakdown of the germinal vesicle, are ineffective if the eggs are first treated with citrate. There is thus support for the theory that stimulation to be effective must involve a calcium release from the cell cortex. It is rather easy to understand why ultra-violet radiation might cause a breakdown of a calcium proteinate gel with consequent release of calcium, for this is consistent with various types of earlier work both on protoplasm and on proteins (for a review of this literature, see Heilbrunn and Mazia, 1936). On the other hand, it is rather paradoxical to assume that immersion of a cell in pure sodium or potassium chloride solution would cause a release of free calcium to the cell interior. However, there is real evidence that this type of phenomenon does actually occur (see Heilbrunn, 1937, Chapter 33).

TABLE IV

Nuclear Breakdown resulting from Irradiation in Sea Water following Citrate Treatment

Immersion time in 0.35 M Na citrate	Immersion time in sea water before irradiation	Percentage breakdown following irradiation
minutes	*minutes*	
6	4	92–95
6	4	98
6	4	98
6	10–10½	99
6	10–10½	100
6	10–10½	98
6	10–10½	100
6½	3½	97

If *Nereis* eggs are immersed in isotonic calcium chloride solutions (0.29 molar) there is no breakdown of the germinal vesicle. Magnesium solutions are also without effect. This apparently means that the calcium ions are unable to penetrate the cell with any rapidity, although this is not the only possible explanation. In the case of the clam *Mactra*, some unpublished experiments of Miss R. A. Young show that activation (which in this egg also involves germinal vesicle breakdown) follows immersion in calcium chloride solutions. (See also earlier work of Dalcq, 1925, 1928; Hörstadius, 1923; Hobson, 1928; Pasteels, 1935.) From these studies with marine eggs we can conclude that for some, calcium chloride solutions are stimulating agents, whereas for others calcium chloride solutions are without any very apparent effect. This is entirely comparable to the situation with respect to smooth muscle. Thus Tate and Clark (1922) showed that calcium caused a contraction of the uterine smooth muscle of the rabbit and cat, but had no effect on similar muscle in the guinea-pig and rat.

In the case of *Nereis*, the eggs become sensitive to calcium chloride solutions and to a lesser extent to sea water, following immersion in sodium citrate solutions. Thus if eggs were immersed in 0.35 molar sodium citrate for 60–65 minutes and then placed in 0.29 molar calcium chloride, 80 to 100 per cent of them showed germinal vesicle breakdown (four experiments gave percentages of 80, 100, 92, 82). It almost appears as though the loss of calcium from the cell cortex makes it possible for calcium to enter the cell more freely. It is possible that there may be a relation between this phenomenon and the calcium paradox as observed in invertebrate hearts (see, for example, Chao, 1934).

Our results lead us to conclude that the breakdown of the germinal vesicle in the *Nereis* egg is the result of a release of calcium from the cortex. Previously Dalcq had suggested that the calcium ion was responsible for the breakdown of the germinal vesicle in the starfish egg, but he offers no clear explanation as to why sodium or potassium should act like calcium, either in the starfish egg or in other eggs. (See Dalcq, 1925, 1928; also Pasteels, 1935.) Our results offer an interpretation of this difficulty. As to the details of the mechanism involved in the disappearance of the nuclear membrane, we are still at a loss. Presumably, calcium ion initiates a series of changes and these changes eventually cause nuclear breakdown. There is obviously more than a mere action of calcium on the nucleus, for when eggs are crushed in sea water, the germinal vesicle does not break down.

Summary

When the *Nereis* egg is stimulated either physically by ultra-violet radiation, or chemically by immersion in sodium or potassium chloride solutions, the germinal vesicle breaks down. This response to stimulation is prevented if the eggs are previously immersed in sodium citrate solutions. The results are in agreement with the calcium release theory of stimulation.

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STUDIES OF THE MITOTIC FIGURE. VI. MID-BODIES AND THEIR SIGNIFICANCE FOR THE CENTRAL BODY PROBLEM

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INTRODUCTION

Purpose of the Study

Mid-bodies have received less attention than any other component of the achromatic figure. In many animal cells they appear, during the time of division, as deeply staining thickenings on the fixed spindle fibers, in the equatorial plane. They are generally regarded as in some way comparable to the cell-plate of plants—possibly a vestigial homolog (Wilson, 1928, pp. 144 and 159; Sharp, 1934, p. 169). Flemming (1891) first suggested this concept.

In a previous study of central bodies in brain cells of *Squalus* embryos, in which considerable attention was also given to the mid-bodies, I reached the conclusion that in this case they are nothing but accumulations of dye at places where the spindle materials have been pinched together by the division furrow, and have no existence as an individualized cell component. The question was raised: "Whether or not mid-bodies generally will eventually be explained in terms of focalization phenomena remains to be seen" (1933*a*, pp. 177–178). This problem is the subject of the present paper.

Discussion of the Term "Focal Body"

Before presenting the data it is advisable to clarify further the concept *focal body* or *focalization phenomenon* which I have discussed previously (1932, pp. 181–182; 1933*a*, p. 177; and 1933*b*, pp. 233–235).[1] These terms are used to describe a structure of the achromatic figure which appears to be produced as a result of the convergence to a common center of either spindle materials, aster materials, or both. If this assumption is correct, there are three possible classes of focal bodies.

Living Focal Bodies.—These probably exist in the living cell as minute pools of spindle or aster materials at centers of focalization, and are preserved by fixation. The situation here may be somewhat like that which occurs when thin lines of a substance of relatively low viscosity are placed upon a piece of glass in a converging configuration: a pool of material forms where the lines coalesce at the center, the size of the focal body thus produced depending upon the number and thickness of the lines, and the viscosity of the substance. Such a body is non-radial and more or less demarked, but although it looks very different from the surrounding radial area and appears to be an individualized structure, it is actually only the inner ends of the lines which have fused.

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[1] Some of these studies, referred to here and later, were carried out with the assistance of other investigators, as indicated in the bibliography.

Coagulation Artifact Focal Bodies.—According to the hypothesis presented here and in earlier papers, these do not exist in the living cell but are created by the act of coagulation. It is suggested that the process of fixation may effect a breakdown of the converging materials at the focal center and form a body, the structure of which is determined largely by the coarseness of the fixed rays and fibers. As I have shown in previous studies, the comparative coarseness of such materials is modified by the technique—the fixative used or the environmental condition prior to fixation. For example, in eggs of *Echinarachnius* (1928, 1929*b*, and 1929*c*) and *Chætopterus* (1932 and 1933*b*) there is a definite relationship between the structure of bodies at centers of focalization and the coarseness of the fibers and rays, as determined by the technique. An analogy to this situation is found in a model similar to the one described above, but employing material of such a composition that the converging lines do not coalesce at the point where they meet. Upon subjecting this model to a given treatment (the equivalent of the fixation procedure), the character of the material is so changed that the lines coalesce at their meeting-point and a focal body is formed.

Staining Artifact Focal Bodies.—These are only accumulations of dye at the focal centers of some fixed mitotic figures, such as I demonstrated in brain cells of *Squalus* embryos (1933*a*). An analogous situation occurs if a model is made in which a large number of delicate threads are attached to the edge of a circular frame and brought together at the center. When such a model is dyed, the focal area takes more stain than the peripheral region, owing to the aggregation of threads there. If this model is viewed at the proper distance (duplicating the effect produced when stained mitotic figures are studied with a microscope) the central region may in some cases look like a sharply demarked body, depending upon the number of threads, the chemical nature of both threads and dye, whether they are thick or thin, their surface rough or smooth, and their paths separate or interwoven.

Assuming that the suggestion is correct that the focalization of spindle and aster materials may result in the production of focal bodies, we still know too little about the structure of living spindles and asters, the effects of coagulation, and the staining process, to explain adequately in physico-chemical terms exactly how the bodies are produced. For these reasons the models just described are not intended to give exact parallels of what occurs when focal bodies are formed in mitotic figures; they only illustrate how focalization phenomena may be involved in any configuration of converging substances.

Depending upon the type of cell used and the technique employed, such bodies may be minute or large, demarked sharply or vaguely, granular or homogeneous or vesicular in structure, and they may differ in shape. Such variations can often be demonstrated in the same material when different techniques are used.

Focal bodies, however, have certain characteristics by which they can be surely differentiated from typical cell components. They exist only at places where spindle and aster materials come to a focus. They arise only as such places of focalization are formed, and disappear when such areas disintegrate; they therefore do not maintain continuity from cell to cell. They change shape as the focal area changes shape, and often become double when the focal area elongates. They may vary widely in structure from one cell cycle to another in the same organism, or they may vary in the same cell cycle in closely related organisms; sometimes, on the same slide, they show differences from cell to cell which are identical as to species, cell cycle, mitotic phase, and the method used. They are usually unstable in structure and easily modified by variations in the technique. In general their morphology is related to the size of the mitotic figure and the coarseness of the fixed focalized materials.

In all of these respects focal bodies differ markedly from such cell components as the centriole-blepharoplasts of spermatocytes and Protozoa, the diplosomes of vertebrate cells, and other similar structures, which have undoubted existence in the living cell as typical individualized cell components, and in some cases can be seen in the living condition. They exist regardless of whether areas of focalization are present or absent, and they exhibit great stability of structure despite wide variation in the technique.

Hence the suggestion is made that at centers of focalization, structures are formed which are often minute, homogeneous, sharply demarked, and look like typical cell components, but are actually nothing more than the result of the convergence of spindle and aster materials to focal areas, i.e., *focal bodies* or *focal phenomena.*

METHODS

The behavior of mid-bodies was studied in eggs of *Cerebratulus*, *Cumingia*, *Nereis*, *Chætopterus*, and *Asterias*, during the formation of both polar bodies, during first cleavage, and also in cells of blastulæ; in *Arbacia* eggs, during the first three cleavages as well as in blastulæ; in brain cells of *Squalus* embryos; and in primary spermatocytes of *Romalea*.

Many fixatives were used and the cells were exposed to various environmental modifications prior to fixation. They were sectioned at a thickness of 5 μ and stained with Heidenhain's hæmatoxylin. In the case of eggs mid-bodies were studied only in those which happened to be sectioned in a plane passing through both the long axis of the mitotic figure and the animal pole, since only in such sections are the relations between the structure of mid-body and spindle shown clearly. In each case an adequate number were examined to ascertain the extent of variation occurring in mid-body structure and associated spindle remnant.

In order to check the conclusions reached by these original studies I also examined a considerable portion of the pertinent literature—almost two hundred papers—to be sure that no important types of mid-bodies described by other workers should be overlooked. In most of these investigations the author was not interested in the mid-bodies; in many cases they are not even mentioned in the paper, although they are shown in the drawings. In only a few studies do the figures delineate the detailed changes from their origin to their disappearance.

The illustrations of the present paper, whether original or reproduced from the literature, show all types of mid-bodies found, typical as well as atypical.[2]

THE BEHAVIOR OF MID-BODIES

Conditions Under Which Mid-bodies Are Present

Mid-bodies arise only when a remnant of the spindle still exists when cell division occurs; this is necessarily pinched together by the advancing furrows. No mid-bodies are found if, as often happens, the spindle disappears prior to division.

Such contrasting conditions may occur in the same cell type of closely related species, as illustrated in cleaving eggs of Echinoidea. In a series of experiments, done in collaboration with Dr. G. H. A. Clowes and Dr. M. E. Krahl, I studied *Arbacia* eggs under about 75 experimental conditions, in an attempt to analyze the relation between modifications of respiration and the behavior of the mitotic figure. Usually the eggs were fixed in Bouin's fluid, but a number of other reagents were used. Mid-bodies are present (Fig. 5) with a spindle remnant (with rare exceptions when the material is very abnormal and both are absent). Boveri (1901) illustrates a similar condition in cleaving eggs of *Echinus* (Fig. 59). On the other hand, *Echinarachnius* eggs were also studied, using about 20 modifications of the technique, and the situation is in contrast to that in *Arbacia* eggs: the spindle disintegrates before division occurs and mid-bodies are not found. *Toxopneustes* eggs (Wilson and Leaming, 1895) also show neither body nor remnant. Here, therefore, are eggs of four species of Echinoidea, two with mid-bodies associated with a focalized spindle remnant, and two with neither.

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[2] Those illustrations taken from the literature (Figs. 13–69) were for the most part reproduced by photographing the drawings of the original papers. In four cases, however (Figs. 14, 62, 65, and 66), photographs were made of illustrations, redrawn from the originals, in Wilson's *The Cell in Development and Heredity*.

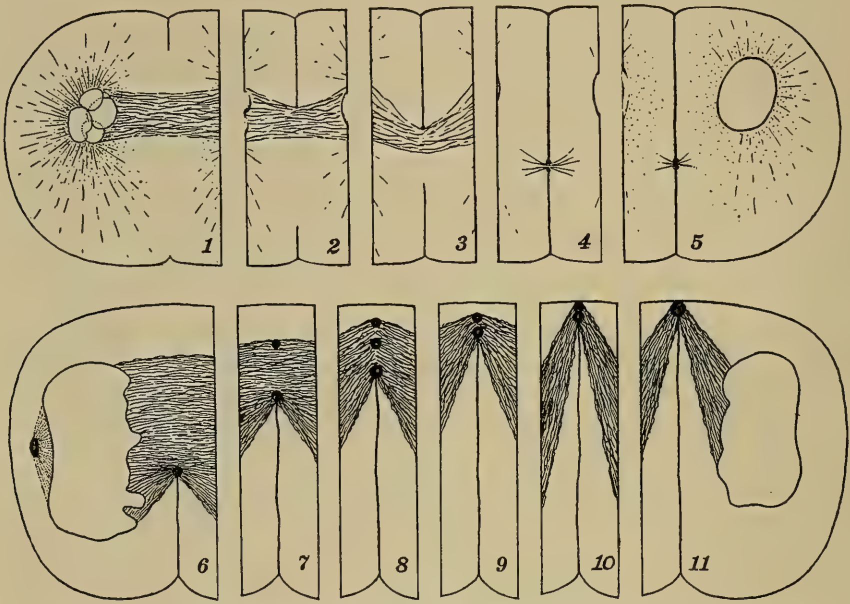
The same relation between the presence and absence of a spindle remnant and the presence and absence of a mid-body may exist even in the same material, under different experimental conditions. When *Arbacia* egg-sets are run at various temperatures, those which cleave at temperatures from 10° to 25° C. show both mid-body and spindle remnant, but at 30° neither is present.

As just noted, a mid-body does not arise unless a remnant of the spindle is present when division occurs. Conversely, when a remnant is present, a mid-body is practically always formed, but a few exceptions to this are reported in the literature. For example, in the spermatogonia of *Enteroxenos* (Fig. 55), the remnant is present without the body. Occasional instances like this are not surprising in view of the fact that the behavior of mid-bodies is so variable, depending upon species, cell cycle, fixation, and depth of stain. Scarcely a single generalization can be made about them to which some exceptions cannot be found.

History of Mid-bodies

There are a number of divergent structural types of mid-bodies, and their history differs. One type is illustrated in *Arbacia* eggs. In this case first cleavage begins with the appearance of a furrow at the animal pole (Fig. 1), but no mid-body arises until the furrow has advanced far enough into the egg to impinge upon the spindle; and the body which then forms at its tip is just a faint thickening (Fig. 2). Another furrow appears at the opposite pole about a minute later, and a similar body arises at its tip when it too presses upon the spindle. Meanwhile the first furrow has advanced more deeply and its body enlarges. By the time the two furrows are ready to touch each other, a mid-body is present at the end of each, and when contact is made these bodies join to form a dumbbell-shaped structure which undergoes no further change until it disappears when the spindle remnant fades (Figs. 3–5).

Another type is illustrated in the brain cells of *Squalus* embryos, in which division occurs from one side only. A mid-body appears at the point where the spindle is first compressed by the approaching furrow. As it advances, other bodies arise, their size and number differing from cell to cell, but the end result in all is a single body at the opposite side of the cell, where the spindle is finally focalized. It too disappears when the spindle disintegrates (Figs. 6–11).



FIGS. 1–11. Successive stages in the formation of mid-bodies. Figs. 1–5: *Arbacia* eggs at first cleavage. Figs. 6–11: brain cells of *Squalus* embryos (Fry and Robertson, 1933).

Several studies in the literature illustrate the history of other types. In erythrocytes of duck embryos (Heidenhain, 1907) a group of nondescript thickenings are aggregated into a centriole-like body (Figs. 16–20). In smooth muscle cells of *Amblystoma* (Pollister, 1932) an atypical elongate mid-body undergoes progressive condensation (Figs. 21–26). And in *Arion* eggs (Lams, 1910) slender elongate thickenings which arise at the middle of the spindle fibers are pinched together into a dense mass (Figs. 27–34).

Although these types differ as indicated, they all have in common a gradual aggregation of mid-body material associated with an increasing focalization of the spindle brought about by the division process.

The final position of the fully formed mid-body is determined by the point where the division furrows make contact. For example, in red blood cells of the duck, the furrows meet at the middle of the cell, and the mid-body lies there (Fig. 19). In *Arbacia* eggs during first cleavage one furrow arises about a minute before the other; their meeting point is therefore considerably off center, and the body lies there (Fig. 5). In *Squalus* brain cells there is only a single furrow arising at one side which presses the spindle against the opposite cell membrane, and the body lies there (Fig. 11).

In most instances mid-body and spindle remnant disappear simultaneously. This is generally true of the materials examined in the present study. It is also shown in the illustrations in the literature, where telophase figures which have both body and remnant are usually succeeded by a drawing of the next mitotic phase without either. In some cases, however, the body persists for a short time after the remnant has disappeared. This point was studied in *Arbacia* eggs at first cleavage. After division is completed, all cells have both mid-bodies and spindle remnants, but about fifteen minutes later, just before the next prophase figure makes its appearance, the remnant is doubtful or absent in about 25 per cent (count of 50), although the bodies are still present in all cells. By the time the prophase figure arises mid-bodies have, with rare exceptions, disappeared. A few cases are also found in the literature where the mid-body is shown as persisting after the spindle remnant has faded, as for example, in red blood cells of the duck (Fig. 20). In other words, if one considers the converging configuration as a whole, the focal area is obviously the most condensed part, and hence this may explain why it occasionally persists longer than the surrounding region of the converging fibers, although it may have been originally created as a result of their becoming focalized.

Configurations of Mid-bodies

The configurations which mid-bodies show during their formation, when cell division is not yet completed and spindle fibers are only partially pressed together, must be distinguished from their final structure, when the aggregation of fibers is fully accomplished. The data here presented refer only to fully formed mid-bodies; the limited number of atypical ones will be described later.

Many mid-bodies are round, smooth, and centriole-like (Figs. 11, 19, 46, 58, and 62); or their surface may be rough (Figs. 56, 57, and 60). Another common type is elongate: it may be ovoid and smooth (Fig. 47), ovoid and rough (Figs. 45 and 59), dumbbell-shaped (Figs. 5 and 61), or irregular (Figs. 48 and 49); it may lie parallel to the major axis of the spindle (Fig. 61) or at right angles to it (Figs. 5, 45, 47, 48, 49, and 54).

In addition to these common forms, other types are found occasionally. The body may be ring-shaped (Figs. 29, 36, and 37), cone-like (Figs. 30 and 52), linear in form (Fig. 26), or it may be composed of thickenings which arise at the middle of the spindle and are then aggregated loosely (Fig. 51) or tightly (Fig. 33). A specialized type is a large irregularly outlined area containing a small vesicle (Fig. 43).

In general the above types are found only when the division process has focalized the spindle to a point or to a very limited area. But associated with a specialized kind of remnant (mitosome) which is not focalized to a point, and persists as a relatively broad band of fibers, is a very different type of mid-body, composed of a row of granules (Fig. 14). In the studies examined this type was found only in spermatogonia and spermatocytes, and not in all of them. Of 15 investigations of spermatogenesis taken at random from the literature, 13 show this unusual remnant and the other two have a fully focalized remnant with a single mid-body (e.g., *Enteroxenos*, Figs. 56 and 57).

In cleaving eggs of *Arion* the mid-body is also a broad band of aggregated spindle fibers, but no granules are present (Fig. 33); and in the polar body cycles of this species there is a similar band associated with a ring-shaped mid-body (Fig. 29).

It is significant that the mid-body which is composed of a row of granules is found only in the specialized broad spindle remnants of male germ cells, whereas the other types which are usually more aggregated, are found with remnants which are focalized to a greater degree.

In some cases the very configuration of the mid-body indicates that it is focalized spindle substances. In cleaving *Arion* eggs, for example, thickenings which appear at the mid-region of the spindle are pressed together by the division furrow into a bundle, which is later bent into a U-shaped figure when the nuclei move from their former position toward the surface of the egg (Figs. 32–34). In cone-shaped bodies, the very form shows that they are spindle substances which have been pinched together (Figs. 30 and 52). And when the atypical, elongate mid-body of smooth muscle cells of *Amblystoma* makes its first appearance it is obviously a condensation of spindle materials (Fig. 22).

The other types—dots, dumbbells, ovoids, rings, lines, and vesicles—which do not appear to the eye to be focalized spindle substances, nevertheless behave like those which do. In general, all types arise after focalization of the spindle has begun, become more aggregated as focalization is completed, and disappear as the spindle remnant disappears. A series of structural groups could be arranged, beginning with the centriole-like type, and passing by gradations to those which are clearly aggregations of spindle materials.

Relation Between Structure of Mid-bodies and Coarseness of Spindle Fibers

When mitotic figures are subjected to various experimental conditions and fixed with different reagents, mid-bodies often vary in size and shape, and the fibers vary in coarseness. The relation between the physical structure of the mid-body and the distinctness of the fixed fibers was studied in primary spermatocytes of *Romalea* and in cleaving eggs of *Arbacia*.

TABLE I

The relation between the size of the granules composing the mid-bodies in primary spermatocytes of Romalea microptera and the coarseness of the spindle fibers. In general, the coarser the fibers, the larger are the granules, but there are various exceptions to this generalization. Forty-eight modifications of the technique were used, and the depth of stain was similar in all. The table shows the percentages of each type of association of mid-body and spindle remnant.

Size of Granules Composing Mid-bodies	Coarseness of Spindle Fibers		
	Vague or Delicate	Coarse	Very Coarse
	per cent	*per cent*	*per cent*
None	8	—	3
Small (0.7 μ)	18	13	21
Medium (1.0 μ)	—	18	15
Large (1.3 μ)	—	—	4

Romalea spermatocytes were subjected to 48 experimental modifications, differing both as to environmental factors prior to coagulation and the fixatives employed. They were all stained to the same depth with Heidenhain's hæmatoxylin. Under usual conditions the mid-body is composed of a row of practically contiguous granules, each about 1 μ in diameter, and the spindle fibers have a certain degree of distinctness. The same situation obtains in spermatogonia (Fig. 14). After modifications of the technique, however, the granules may be absent, small, medium, or large, and they may vary in shape; the fibers may be vague, delicate, coarse, or very coarse.

The relation between variation in size of granules and coarseness of fibers is reported in Table I. In general, the more delicate the fibers are, the smaller the granules. But the presence of very coarse fibers does not guarantee large granules, and those of small size are associated with fibers of all degrees of coarseness.

In *Arbacia* eggs, in the 75 experiments previously noted, the dumbbell-like mid-body is in most cases of usual length—about 1.5 μ—and the small spindle remnant is composed of delicate fibers. But in 6 of the experiments the fibers are unusually coarse and the bodies are larger. In this material too, however, there are a few exceptions: the remnant may have the usual delicate appearance and yet be associated with exceptionally large bodies, or the fibers of the remnant may be very coarse while the bodies are of usual size.

In general, therefore, the size of the mid-body is directly proportional to the coarseness of the spindle fibers, but there are exceptions. These are probably explained by the uncertainties of focalization phenomena—which is another way of saying that we do not know what the structure of the living spindle is, and we know even less about the effects of coagulation upon it, especially at points of focalization. Furthermore, when we are dealing with areas of focalization, we must also remember that slight differences in depth of stain may produce marked differences in appearance.

Variations in Mid-body Structure from One Cell Cycle to Another in the Same Species

Within the cell types of any one species—oögonia, oöcytes, spermatogonia, spermatocytes, large blastomeres, small blastomeres, and the innumerable kinds of somatic cells—there may be wide variation in mid-body structure.

In eggs of *Cerebratulus*, *Cumingia*, *Nereis*, *Chætopterus*, and *Asterias*, the mid-bodies of the first polar body figure are larger than those of the second. In *Arbacia* eggs, also, during the first three cleavages, the average length of the dumbbell-shaped bodies is successively 1.5 μ, 1.4 μ, and 1.2 μ (25 measurements in each case). And in *Cumingia* the diameter of the mid-body at first cleavage is 1.6 μ, but at the second it is 1.0 μ. In all these cases Bouin's reagent was employed. It is probable that differences in the size of the mitotic figure may explain why mid-bodies are larger in the first polar body figures than in the second, and larger in first-cleavage figures than in those following.

In *Nereis* eggs, during the first polar body cycle, the mid-body is round and relatively large, with a rough surface (Fig. 60); during the second cycle it is dumbbell-shaped and small, with a smooth surface (Fig. 61); at first cleavage, however, it is absent (Bouin's fixation). This is probably explained by the fact that a spindle remnant is present when the polar bodies are pinched off, but not when the egg divides.

Crepidula eggs (Conklin, 1902) show striking differences in mid-body structure from cycle to cycle. In first polar body figures it is a ring, which appears as two dots when seen in cross-section (Figs. 35 and 36); in the second, it is a smaller ring with a dot in it (Fig. 37), or just a dot (Fig. 38); in first-cleavage figures it is a large area containing a vesicle (Fig. 43); in third-cleavage figures it is a small irregular blob (Fig. 45), and in fourth-cleavage figures, a minute dot (Fig. 46). The difference in size of the mid-bodies is apparently related to the size of the mitotic figures concerned. The factors which produce rings in some cells and vesicles or dots in others are not as yet understood.

Variation in the structure of mid-bodies is also exhibited in *Enteroxenos* cells in different cycles (Figs. 47–57).

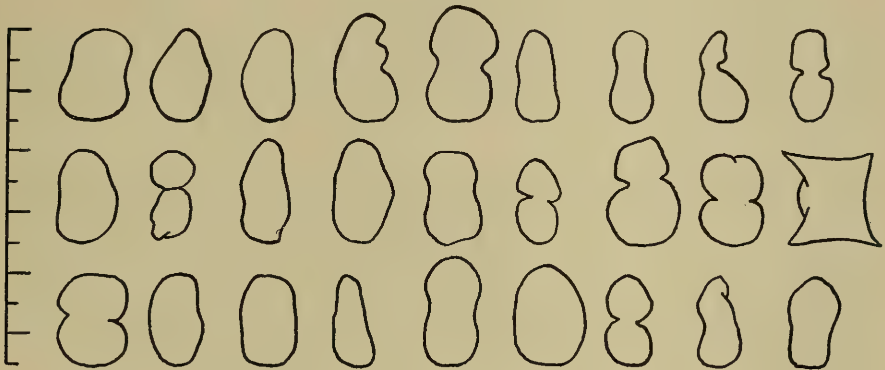


FIG. 12. Variation in mid-body structure in *Arbacia* eggs at first cleavage. Twenty-five bodies selected at random from a single slide are shown in outline and without their accompanying spindle remnants. The unit of the scale is 0.5 μ. All eggs are in late telophase just after cleavage is completed. Bouin's fixation.

Instability of Mid-body Structure

Mid-bodies vary in size and shape from cell to cell on the same slide. Outline drawings of mid-bodies in *Arbacia* eggs at first cleavage (Fig. 12) illustrate the variation in size and contour which may occur in cells at the same stage treated identically and lying side by side on the same slide. The extent of this variation is typical of the materials reported in this paper.

Mid-bodies of oögonia (Figs. 47–49) and those of the second polar body figures (Figs. 52–54) in *Enteroxenos* cells also exhibit variation in shape.

Mid-bodies vary in structure due to the use of different fixatives. In some instances a wide modification of the technique has but little effect upon the structure of the mid-body, as in *Arbacia* eggs at first cleavage (Fig. 5), but in others the effects are considerable, as in primary spermatocytes and spermatogonia of *Romalea* (Fig. 14 and Table I). As a general rule, if fixation demonstrates coarse spindle fibers, the mid-bodies are large; if the fibers are delicate, the bodies are smaller.

Mid-body structure is varied by modifications of environmental factors. In *Arbacia* eggs the size of the mid-body at first cleavage is modified by temperature. At temperatures from 25° to 20° C. the length is 1.5 μ; at 15° C. it increases to 2.8 μ; at 10° and 7.5° it again has an average length of 1.5 μ. There is, however, much greater variation at the lower temperatures than at the higher ones. (Bouin's reagent used; counts of 25 in each case.)

When *Cerebratulus* eggs cleave at 20° C., mid-bodies are present only in about 2 per cent of the cells (Boveri's picro-acetic reagent), and are less than 1 μ in diameter; but when these eggs are allowed to develop at 15° C., and are fixed in the same manner, mid-bodies over 2 μ in diameter are present in about 10 per cent of the eggs. (Counts of 50 eggs.)

Mid-bodies may be present in some cells and absent in others prepared in an identical manner. A given egg-set may be fixed under usual laboratory conditions and run up in the regular manner, yet cells on the same slide and at the same mitotic phase may show mid-bodies and remnants in some cases and neither in others. For example, *Cerebratulus* eggs, as just noted, have only 2 per cent with mid-bodies and spindle remnants at first cleavage. *Cumingia* eggs at first division show 18 per cent (Bouin's fixation), and *Arbacia* eggs show 30 per cent when dividing at 7.5° C. (Bouin's reagent). These percentages are based on counts of 50 eggs. Various similar examples could be cited.

But no matter how the experimental technique is modified, a mid-body is present only when there is a spindle remnant, with the rare exceptions previously mentioned (pp. 569 and 571). How shall we explain, then, the presence of a spindle remnant in some eggs and its absence in others which are at the same mitotic phase, the two kinds lying side by side on the same slide? The probable cause of this variation is an uncontrollable factor in the fixation process: eggs are added to the reagent in a certain amount of sea water, necessarily causing some dilution of the fixative. Each egg is coagulated within the first second after exposure to the reagent (based on a study of *Chætopterus* eggs (Fry, 1932, pp. 173–176)), but several seconds are required for the egg suspension and the fixative to mix completely.

Some of the eggs are thus coagulated by the reagent at full strength and others at various degrees of dilution. In *Chætopterus* eggs such uncontrollable dilutions of the fixative produce marked differences in the structure of astral rays and central bodies (Fry, 1932, pp. 161–167). This factor may also operate in the case of mid-bodies and their associated spindle remnants, and may explain their instability of structure, their presence in some cells and absence in others on the same slide and their differences in size and contour from cell to cell.[3]

Mid-bodies in Protozoa

Mid-bodies are usually absent in Protozoa. In the majority of species mitosis is intranuclear, the nucleus separating into two parts while the membrane remains intact. The morphological details of the process of separation vary widely: in many cases the connecting strand, prior to the final separation, is long and thin, while in others it is relatively broad; in some the spindle fibers are visible up to the time the break occurs, and in others they disappear before then. But in most species there is no special point of focalization and no mid-body. There are a few exceptions, however, where the mode of nuclear constriction aggregates the spindle fibers to a point or a limited area, and these may have mid-bodies, as in *Ceratium* (Lauterborn, 1895) and *Coccidium* (Schaudinn, 1900).

In the occasional instances where the nuclear membrane disappears and the division figure lies in the cytoplasm, a mid-body may arise as the spindle is focalized by the process of cell division, exactly as in eggs of Metazoa. This is illustrated in *Acanthocystis* (Schaudinn, 1896). But if cell division does not occur at the time when such a cytoplasmic figure is present, and the spindle is hence not focalized, there is no mid-body, as shown in *Monocystis* (Muslow, 1911).

Atypical Mid-bodies

The mid-bodies thus far described arise without exception only as spindle fibers are focalized. There are, however, several cases illustrated in the literature where thickenings appear either just prior to the process of focalization or in its complete absence.

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[3] With the assistance of Dr. George Child, an attempt was made to produce mid-bodies artificially. Somewhat in advance of the time when focalization of the spindle occurs naturally, eggs of *Arbacia* and *Chætopterus* were individually constricted with a glass hair in a plane passing through the middle of the late anaphase spindle, in order to focalize it artificially. They were fixed instantaneously while the needle was still in position, and then run up individually. The experiment failed, because the protoplasm was rendered completely hyaline by the manipulation, in the plane through which the needle passed, and no structure of any kind could be seen.

In *Arion* eggs (Lams, 1910), both in polar-body and first-cleavage figures, thickenings arise at the middle of the spindle during anaphase, before division has begun. Thereafter these are aggregated in an orthodox manner into a bundle-like body when division occurs (Figs. 27–34). A similar situation is found in spermatogonia of *Blaps* (Nonidez, 1920).

In spermatocytes of *Llaveia* (Hughes-Schrader, 1931) the spindle is composed of tubes, each associated with a tetrad or a dyad. Soon after the chromosomes separate, during anaphase, the central part of each tube shrinks to a cord-like structure. At this time in some tubes, or a little in advance of the shrinking process in others, mid-bodies make their appearance—one in tubes associated with dyads, two in those with tetrads. A little later the tubes are pressed together by the division furrows and coalesce, the mid-bodies still maintaining their identity (Fig. 63).

In developing *Drosophila* eggs (Huettner, 1933) mid-bodies arise at the middle of the disintegrating spindle during telophase, in the complete absence of cytoplasmic division (Fig. 64). This also occurs in maturating eggs of *Aspidiotus* (Schrader, 1929).

These rare cases which, with the exception of *Arion*, I found only in cells of insects, do not invalidate the fact that the great majority of mid-bodies arise only in connection with the focalization of spindle fibers. No explanation can be given for these exceptions, but such behavior is not surprising in disintegrating gelatinous material.

Certain other cases reported in the literature might be regarded as atypical, but they are only additional examples of the instability of mid-bodies. For example, Buchner (1915, p. 28) illustrates in *Ascaris* eggs a mid-body of irregular shape without any spindle remnant, whereas Carnoy and Lebrun (1897, Plate II), who studied the same material and used a similar, though not identical reagent, illustrate neither body nor remnant. Such different results may be due to differences in the reagents employed, or, if the mid-body here is a staining artifact, to variations in the depth of stain. Furthermore, where Buchner shows it without an associated spindle remnant, this may be one of those occasional cases where the body persists after the remnant has disappeared.

Discussion

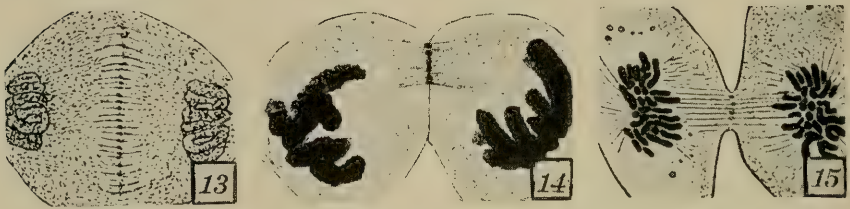
The Nature of Mid-bodies

The foregoing facts indicate that mid-bodies are phenomena of focalization. They do not arise in cells in which the spindle disintegrates prior to the time of division; and in those in which the spindle is still present at that time, mid-bodies appear only as the fibers are focalized by the process of cell division; they disappear, with rare exceptions, when such areas of focalization disintegrate. Their wide variability as to mode of formation is associated with the manner in which the spindle fibers are aggregated. Their final position in the cell is determined by the point at which the spindle remnant is pinched together by the division furrows. They often show variability in appearance from species to species, from cell cycle to cell cycle, and even from cell to cell on the same slide. They are frequently modified by differences in the fixatives employed, or variations in environmental factors prior to fixation, and such differences are usually related, with exceptions, to the size of the mitotic figure concerned, and the coarseness of the spindle fibers. In many cases their structure is unquestionably nothing but aggregated spindle substances; and it appears that such focalization phenomena may take many forms, sometimes even simulating centrioles.

Three classes of focal bodies were mentioned earlier: living focal bodies, coagulation artifact focal bodies, and staining artifact focal bodies. A number of experiments were carried out to determine to which of these categories mid-bodies belong. Some of them undoubtedly exist as a delimited structure in the coagulated cell. In these cases, when the materials are slowly destained, the result being closely observed at every stage by the use of a high power water-immersion objective, it is seen that the bodies retain the dye (Heidenhain's hæmatoxylin) to a greater extent than any other cell component, but they too finally yield it. During the destaining process they grow lighter in color but show no change in size. When the fully destained slides are run up and studied with an oil immersion objective, the bodies are still seen distinctly as refringent structures, although they are colorless. Examples of this class of focal bodies are found in the first polar-body cycle in *Nereis* (Fig. 60) and in cleaving *Arbacia* eggs (Fig. 5). Whether they existed in the living cells as minute pools of fiber material at the focal center (living focal bodies), or the living point of focalization had no such structure and the body was produced by fixation (coagulation artifact focal bodies) cannot be determined because no structure can be seen in the living spindle.

Other mid-bodies, though sharply demarked and sometimes looking exactly like centrioles, are clearly nothing but accumulations of dye at focal points. If the materials are heavily stained with Heidenhain's hæmatoxylin, the bodies are relatively large; if stained in the ordinary manner, they are smaller. If these bodies are gradually destained, while watching the process, they do not lose their color while maintaining their characteristic size, but throughout the process they remain intensely black and become progressively smaller until they disappear. When such completely destained preparations are run up and studied in the usual way they show fibers which come to a point without the presence of a body. This is the case for example, in brain cells of *Squalus* embryos which have a centriole-like mid-body (Fig. 11).

The usual hypothesis that mid-bodies of animal cells are related in some way to the cell-plate of plant cells is not consistent with the behavior of mid-bodies as here described. The cell-plate is associated with a broad spindle which has distinct fibers in the mid-region (Fig. 13), whereas the mid-body is associated with a disintegrating spindle, and only after its fibers have been focalized. In most instances, regardless of their diversity in shape, mid-bodies are single structures which do not resemble cell-plates in any way. In certain cases, however, there is enough resemblance to have given rise to the hypothesis. The mid-body which is composed of a row of granules simulates the fixed cell-plate to some extent, but the differences are marked (Figs. 13 and 14). Also, there is occasionally a brief phase during the formation of a single mid-body when several granules are present (Fig. 15) that calls to mind the cell-plate. In general, however, it seems that the mid-body and the cell-plate have nothing in common.



Figs. 13–15. Resemblances between the cell-plate of plant cells and mid-bodies of animal cells. Fig. 13: cell plate in pollen mother cells of *Fritillaria* (Strasburger, 1888). Fig. 14: fully formed mid-body in *Romalea* spermatogonia (Wilson, 1928). Fig. 15: early stage during formation of mid-body in epithelial cells of the salamander lung (Flemming, 1891).

But regardless of the relation of the mid-body to the cell-plate, the question may be raised as to whether or not the mid-body is a true cell component playing some rôle in the process of cell division and the consequent focalization of the spindle. May it be a causative factor and not just an effect of focalization? The findings of this study do not support such an interpretation. (1) In some cases the mid-body is only an accumulation of dye. (2) *Arbacia* eggs cleave whether mid-bodies are present (at temperatures from 10° to 25° C.) or absent (at 30° C.), indicating that they play no essential rôle. (3) This conclusion is also supported by the fact that mid-bodies are present in cleaving eggs of some species of Echinoidea but not in others. (4) And all the other data presented in this investigation make it highly improbable that we are here dealing with a cell component which plays a causative rôle.

Cells exhibit other areas of focalization—aside from central bodies which will be discussed in a moment—that behave much like mid-bodies. In his classic study of *Crepidula* eggs Conklin (1902) illustrates an unusual case. During anaphase of the second maturation division the minute centriole becomes a vacuole, while the centrosome enlarges. For a very brief period, during middle anaphase, a number of minute bodies appear, each associated with a bundle of spindle fibers (Figs. 67–69). No such bodies arise during the first polar-body cycle in this species. In *Chætopterus* eggs, however, such phenomena occur during the first maturation division but not in the second (Mead, 1898, Plate 17). In *Ascaris* eggs (Carnoy and Lebrun, 1897, Plate I) they occur in both cycles. Bělař (1928, p. 35) illustrates a somewhat similar situation in *Monocystis*.

Another possible phenomenon of focalization is shown when protoplasm and certain artificial emulsions are fixed with reagents which demonstrate foam structure (Bütschli, 1894). Minute bodies are frequently found at the points where the lines of the foam structure meet (Figs. 65–66) and these may be focal bodies.

A still different phenomenon of focalization is illustrated in telophase of the first polar body cycle of *Arion* eggs (Fig. 30). By the time the center of the old aster has disintegrated two new central bodies and asters have appeared within it. The rays of the old aster are most aggregated about this center, where they form a dark, diffuse, ring-like area of focalization.

The Significance of Mid-bodies for the Central Body Problem

If focalization of the middle of the spindle may result in the formation of structures which in some cases simulate individualized cell components, it is in order to examine the situation at the ends of the spindle, where similar focalization areas occur, of both rays and fibers, or fibers alone.

In certain cases the similarity in appearance between mid-bodies and central bodies is so marked that it compels attention. For example, Conklin (1902, p. 43)[4] notes the similarity in first-cleavage figures of *Crepidula* eggs: "This mid-body is for all the world like a centriole with its surrounding centrosome and aster, and recalls Watasé's (1893) comparison of the mid-body to an intercellular centriole. This apparent resemblance is still further supported by the fact that the mid-body in this case becomes a hollow sphere before it finally disappears, just as the centriole does. The mid-body is surrounded by a darkly staining substance which resembles the centrosome substance" (Figs. 39–44). In short, the structural changes which occur in cleaving *Crepidula* eggs at the ends of the spindle during the early history of the mitotic figure are later repeated at the middle of the spindle when it is focalized by the division process. Furthermore, mid-bodies and central bodies are again identical in appearance at fourth cleavage, though here both are minute dots (Fig. 46). Such a similarity of appearance occurs frequently: in *Squalus* brain cells (Fig. 6), in leucocytes (Fig. 58) and connective tissue cells (Fig. 62) of the salamander, in erythrocytes of the duck (Fig. 19), in cleaving *Echinus* eggs (Fig. 59), and in many other cases.

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[4] In using this quotation I have taken the liberty of substituting modern terminology (according to Wilson, 1928, p. 675) for that used in the original paper. The modern term *centriole* is used instead of the old term *centrosome* to indicate a minute, sharply demarked body; the modern term *centrosome* is used instead of the old term *sphere* to describe a larger less sharply demarked area which surrounds the centriole or exists alone; the term *central body* is a general one which includes either or both of the others.

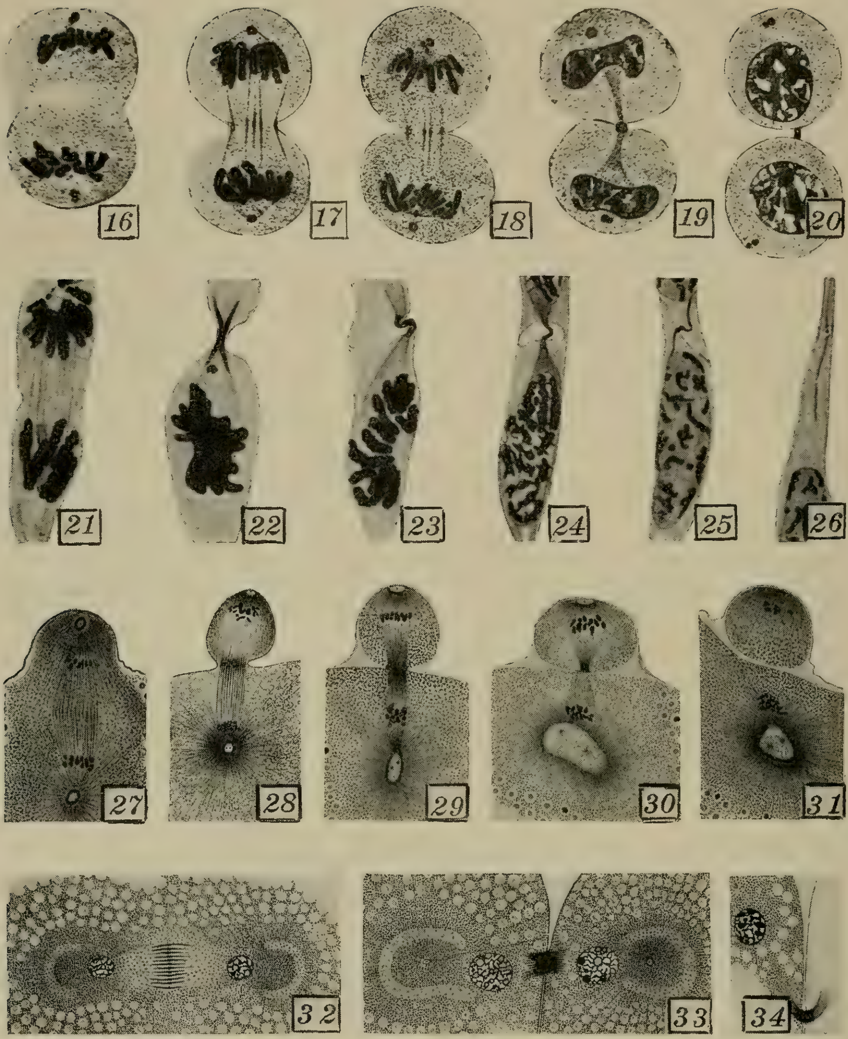
There are numerous instances, however, where the two bodies differ completely in appearance. The sharply demarked, minute, dumbbell-like mid-body in cleaving *Arbacia* eggs (Fig. 5) bears no resemblance to the large non-demarked granular central body. The elongate mid-body in the smooth muscle cells of *Amblystoma* is unlike the typical centriole (Figs. 21 and 26). In the first maturation division of *Arion* eggs the mid-body is a band of fibers surrounded by a ring, in contrast to the minute dot-like centrioles (Fig. 29); during first cleavage, however, it is a similar band but without a ring, whereas the central body is a large area concentrically differentiated and without centrioles (Fig. 33). *Crepidula* eggs during maturation (Figs. 35–38) and *Enteroxenos* cells at various cycles (Figs. 47–57) also exhibit differences in appearance between mid-bodies and central bodies, and many other examples could be cited.

The uncertainties of focal phenomena may explain why mid-bodies and central bodies look alike in some cases and different in others, the instances of dissimilarity being somewhat more numerous. But the significant point is not whether mid-bodies and central bodies *look* alike, but whether or not they *behave* alike.

In my studies of central bodies in various cell cycles of several species, some of which were used in the present investigation of mid-bodies, I demonstrated that the structure of the central body in these cases is related to the structure of the rays or fibers. In cytasters of artificially activated *Echinarachnius* eggs (1928), where no spindle is present, a central body occurs only when rays reach the center, regardless of how distinct the rays are peripherally. When a spindle is present without asters, as in *Squalus* brain cells (1933*a*), the structure is again related to the coarseness of the fibers. When the history of the central body is followed from the beginning to the end of a mitotic cycle, as it was in cleaving *Echinarachnius* eggs (1929*a*) and *Squalus* brain cells (1933*a*), it is apparent that the central body undergoes changes related to the coarseness of the converging rays and fibers, and their general configuration. The continuity of the bodies from one cell cycle to another cannot be demonstrated; they arise as areas of focalization arise, and disappear as such areas disintegrate, even though the peripheral region still has distinct rays. When, at any given mitotic phase, rays and fibers are modified by the use of different fixatives or environmental factors, the structure of the central bodies is changed, as shown in *Echinarachnius* eggs at metaphase of the first-cleavage figure (1929*b* and 1929*c*). This relationship is also shown with unusual clearness in *Chætopterus* eggs (1932 and 1933*b*). Here, furthermore, when supposedly typical centrioles are demonstrated, it is found that they differ in size and contour from cell to cell, at the same mitotic phase, on the same slide. Hence the conclusion was reached that in these cases the supposed central bodies are phenomena of focalization: staining artifacts in *Squalus* brain cells, and coagulation artifacts in the others.

The technique used in studying the mid-bodies reported in the present paper was the same as that employed in the investigations of central bodies just noted. Many fixatives were used to modify the structure of the fibers, in order to determine the effects of such modifications upon the structure of the bodies; for the same reason, cells were also subjected to abnormal environmental conditions prior to fixation. In each case the sample studied was large enough to determine the extent of structural variation, and all classes were reported and considered in arriving at the conclusion.

If focalization phenomena are involved in the formation of both mid-bodies and central bodies, it is rather surprising that in mitotic figures which have asters, those bodies occurring at the middle of the spindle where only fibers are present, should ever look like those occurring at the spindle-ends where both fibers and rays are concerned. But, as previously mentioned, in some cases they may be identical in appearance, and in others completely dissimilar. At present it is as impossible to explain this fact as it is to explain why, for example, the mid-bodies of *Crepidula* eggs are ring-like during polar body formation, but vesicle-like at first cleavage, and centriole-like at fourth cleavage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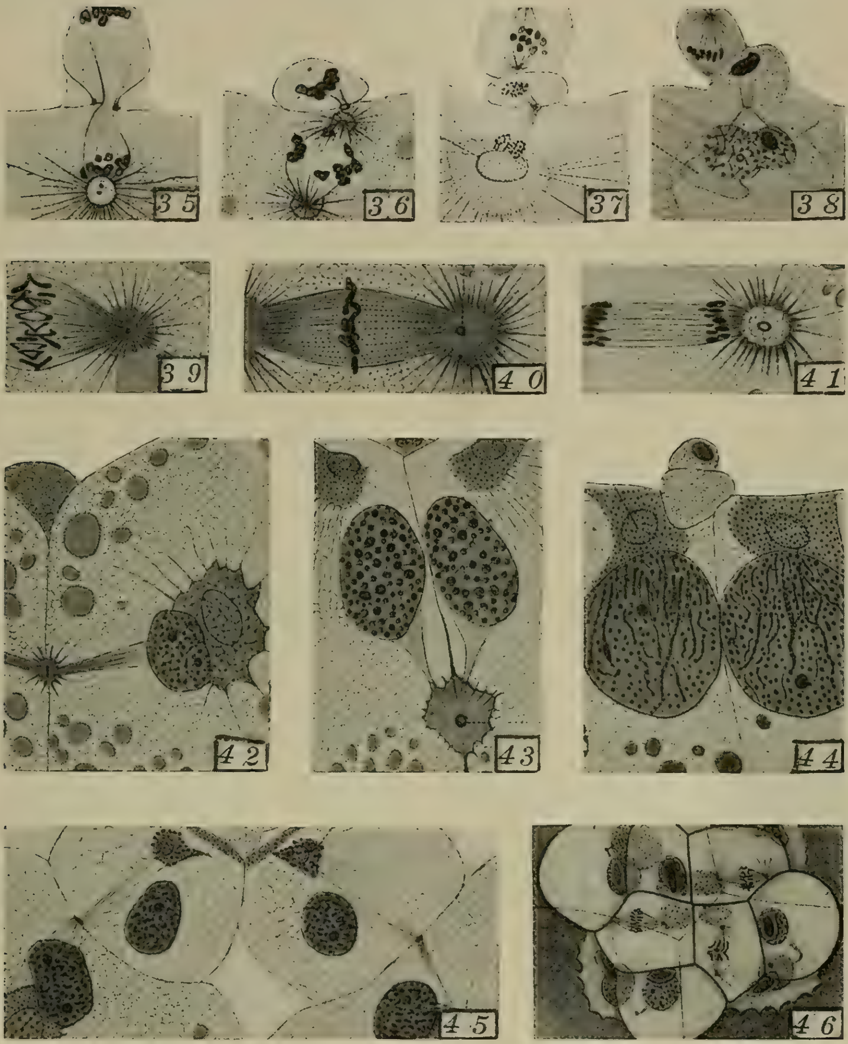
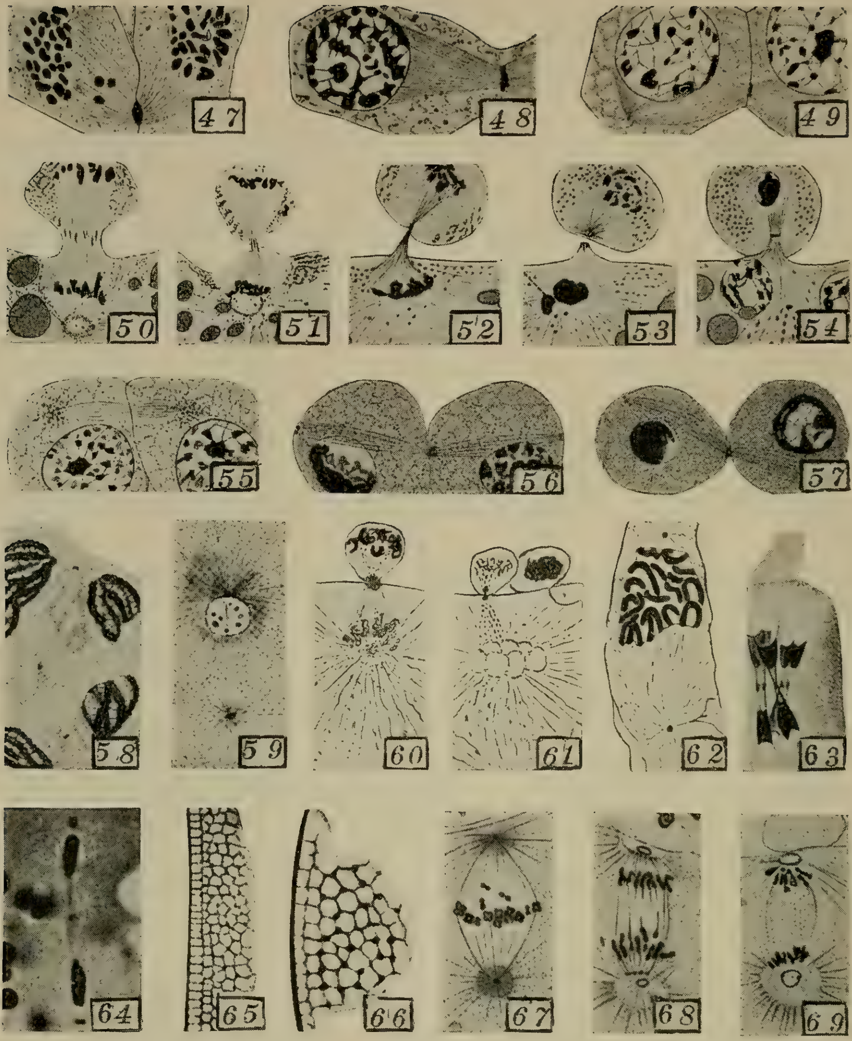
FIGS. 16–34. Successive stages in the formation of mid-bodies. Figs. 16–20: erythrocytes of duck embryos (Heidenhain, 1907). Figs. 21–26: non-striated muscle cells of *Amblystoma* larvae (Pollister, 1932). Figs. 27–34: first polar bodies and first cleavage in *Arion* eggs (Lams, 1910).



FIGS. 35–46. Mid-body structure and central body structure in various mitotic cycles of *Crepidula* eggs (Conklin, 1902). Figs. 35–36: first polar bodies. Figs. 37–38: second polar bodies. Figs. 39–44: first cleavage. Fig. 45: third cleavage. Fig. 46: fourth cleavage.



FIGS. 47–69. Mid-bodies in various mitotic cycles of *Enteroxenos* cells (Bonnevie, 1906). Figs. 47–49: oögonia. Figs. 50–51: first polar bodies. Figs. 52–54: second polar bodies. Fig. 55: spermatogonia. Fig. 56: primary spermatocytes. Fig. 57: secondary spermatocytes. Mid-bodies of various cells. Fig. 58: salamander leucocytes (Bělař, 1928). Fig. 59: cleaving *Echinus* eggs (Boveri, 1901). Figs. 60–61: first and second polar-body figures of *Nereis* eggs (original). Fig. 62: connective tissue cells of the salamander lung (Flemming, 1891). Fig. 63: primary spermatocytes of *Llaveia* (Hughes-Schrader, 1931). Fig. 64: developing *Drosophila* eggs (Huettner, 1933). Bodies occurring at the junction of the walls of alveoli. Fig. 65: fixed eggs of *Sphaerechinus* (Bütschli, 1894). Fig. 66: artificial emulsion of olive oil and NaCl (Bütschli, 1894). Multiple bodies, in addition to central bodies, occurring at spindle-ends during middle anaphase in the second maturation division of *Crepidula* eggs (Conklin, 1902). Fig. 67: early anaphase without bodies. Fig. 68: middle anaphase with bodies. Fig. 69: late anaphase without them.

(Figs. 35–46). If, however, the focalization of fibers or rays may cause the production of focal bodies, it is not surprising that there is variation in their structure according to the size of the configuration as a whole and the coarseness of the converging fibers. But our knowledge of the physical chemistry of such systems is not sufficient for us to explain the structural diversity in focal bodies, from species to species, and cell cycle to cell cycle, in mitotic figures of the same size and distinctness.

If the conclusion of the present study is valid, we cannot accept the presence of a sharply demarked body in an area of focalization—whether at the middle or the ends of the spindle—as evidence that we have demonstrated a true cell component. This is, however, the generally accepted practice. Structures which appear to the eye to be as individualized as chromosomes or plastids may nevertheless be merely ephemeral transient phenomena of focalization—sometimes nothing but focal accumulations of dye, as in both mid-bodies and centrioles in brain cells of *Squalus* (Fig. 6) (Fry and Robertson, 1933). The eye sees such a structure, and the mind accepts it on the basis of its appearance and attempts to imagine its function.

The fact that bodies do not arise at the middle of the spindle until it is focalized by the division furrows should make us alert to possible pitfalls—errors in interpretation—when structures are found at points where fibers or rays or both converge. In this connection it is significant that bodies are not present at the ends of anastral spindles unless the tips are sharply focalized. The blunt, anastral spindles found in some oöcytes and spermatocytes do not have them (with possibly a few exceptions in the latter when centriole-blepharoplasts are present).

If the conclusion of this investigation is correct, the current central body hypothesis must be reevaluated. Focal bodies have probably been confused with such cell components as centriole-blepharoplasts of male germ cells, diplosomes of vertebrate cells, and similar structures of Protozoa and other cell types, which for the most part are concerned with the formation of axial filaments and flagella, no matter what their rôle in mitosis. The great majority of cell types have no such components, and it is yet to be ascertained to what an extent their supposed central bodies are actually artifacts of focalization.[5]

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[5] The data of the present study suggest that the spindle, despite its homogeneous appearance in the living cell, has some kind of linear organization. The very fact that bodies appear in the plane where the division furrow exerts pressure on the spindle indicates that there are differentiated materials there. If the spindle were actually homogeneous, it is probable that its materials would flow to one side or the other when it is pinched together, in which case no focalization bodies would be formed.

RESUMÉ

Mid-bodies were studied in various cell cycles in a number of species; many fixatives were used and cells were subjected to different environmental modifications prior to fixation, to modify the structure of both bodies and fibers in order that the relation between them might be analyzed.

Mid-bodies are found only in cells in which the spindle is still present when division occurs; they are absent in the numerous cases where the spindle disappears before that time. They arise only as the spindle fibers are gradually brought to a focus by the advancing division furrows; they usually disappear simultaneously with the spindle remnant. Their final position is determined by the point where the division furrows meet. There are many structural types: centriole-like dots, blobs, ovoids, dumbbells, rings, cones, lines, rows of dots, and other configurations. Some are obviously nothing but aggregated spindle materials, whereas others look like individualized cell components. Different structural types may occur in the same species in successive cell cycles. They may vary in size and contour from cell to cell on the same slide. They are generally unstable and easily modified by the use of various environmental factors or different fixatives. Such structural modifications are usually related to the coarseness of the fibers.

Mid-bodies are generally regarded as a vestigial homolog of the cell-plate of plants. The present study, however, interprets them as phenomena of focalization or "focal bodies." It is suggested that these are produced as the result of the concentration of spindle or aster materials at points where they are focalized. In some cases mid-bodies are only an accumulation of dye at the focal area; in others they are probably produced by the process of fixation.

If bodies which look like typical cell components can be formed at the middle of the spindle, as the result of the focalization of its materials brought about by the advancing division furrows, similar phenomena may occur at the areas of focalization at the spindle ends where both fibers and rays may converge. Previous studies of central bodies, in some of the same cells used for the present study of mid-bodies, indicate that they too are produced as a result of the focalization of spindle and aster materials. It is, therefore, suggested that in the formulation and development of the current central body hypothesis, focal bodies may have been confused with true cell components such as centriole-blepharoplasts, diplosomes, and similar structures.

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A PHYSIOLOGICAL AND HISTOLOGICAL STUDY OF THE FRONTAL CORTEX OF THE SEAL (PHOCA VITULINA)[1]

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The so-called "motor areas" of the cortex of the cat, dog and bear have been investigated recently with the combined methods of electrical stimulation and histological examination (Langworthy, 1928; Smith, 1933, 1935, etc.). The general pattern, both functional and anatomical, of these three fissiped carnivores is quite similar, although minor variations are distinct. It was therefore of interest to make a comparative study of the harbor seal (*Phoca vitulina*), a pinniped carnivore which is functionally adapted to an aquatic existence and in which the trunk and extremities are very considerably modified (Howell, 1928).

I should like to express my thanks to the Superintendent, Mr. Thomas H. Dorr, and his assistants, particularly Mr. E. C. Barter, at the Boothbay Harbor Station of the United States Bureau of Fisheries, for obtaining the animals and assisting in handling them.

METHODS

Experiments were successfully completed on 6 seals approximately 4 to 5 months old. The animals were in excellent condition and weighed between 18 and 25 kgm. Satisfactory anæsthesia was obtained with Dial fluid (Ciba) 0.3 cc. per kgm. intraperitoneally, supplemented by a few whiffs of ether during the opening of the skull. It was found preferable to keep the body of the seal submerged in a tank of water during the early stages in order to assist respiration.[2] The thin skull was readily removed with trephine and rongeurs. Since the electrically excitable cortex was located behind the eye, the latter was enucleated and the orbital roof removed in the later experiments. The rectal temperature of the animals was kept between 37° and 40° C. by pouring cold sea water over them occasionally.

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[1] The seals were obtained and the physiological observations made at the Boothbay Harbor Station of the U. S. Bureau of Fisheries.

[2] When on land the seal normally breathes rhythmically and regularly. Five to ten minutes after the injection of the dial, respirations became intermittent. Four to five deep breaths were taken, ending in inspiration, following which the nostrils were tightly closed and the breath held for 15 to 40 seconds, the cycle being then repeated. The administration of ether by a cone during three or four of these cycles resulted in regular, slow deep respiration.

Stimulation was bipolar by means of a pair of silver-silver chloride electrodes with an interpolar distance of 2 mm. Current was supplied by the 60 cycle a.c. line through a potentiometer. The effective voltage varied in different animals from $\frac{1}{2}$ to 5 volts. For single shocks a Harvard inductorium, with 3 volts in the primary circuit and the secondary coil set at 7 to 9 cm., was employed. Between observations the cortex was kept moist by application of cotton pledgets wet with warm Ringer's solution.

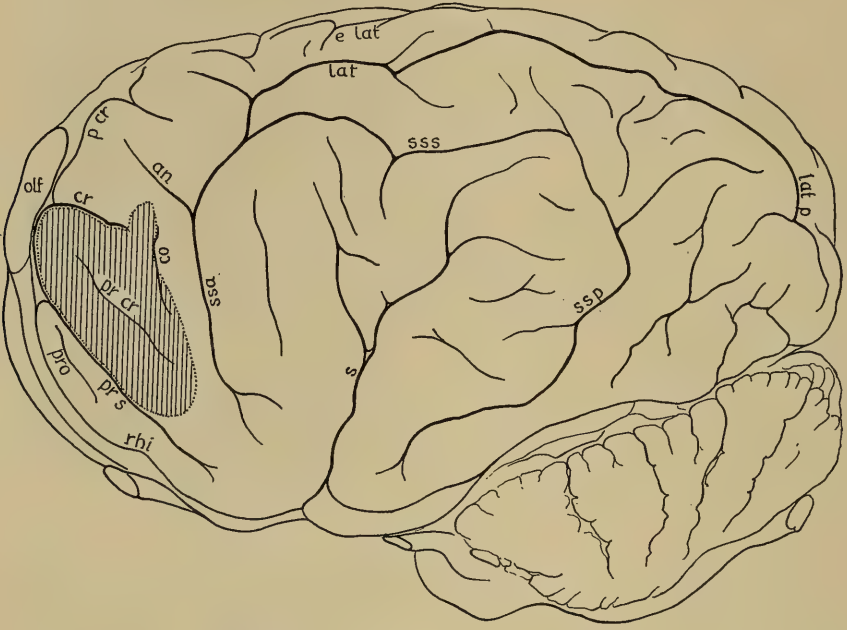


FIG. 1. Outline drawing of a lateral view of the brain of seal 5. × 1.5. The position of the electrically excitable cortex is indicated by the shaded area. *an*, S. ansatus. *co*, S. coronalis. *cr*, S. cruciatus. *e lat*, S. endolateralis. *lat*, S. lateralis. *lat p*, S. lateralis posterior. *olf*, Bulbus olfactorius. *p cr*, S. postcruciatus. *pr cr*, S. precruciatus. *pr s*, S. presylvius. *pro*, S. proreus. *rhi*, Fissura rhinalis. *s*, S. pseudosylvius. *ssa*, *ssp*, *sss*, S. suprasylvius anterior, posterior and superior.

At the end of the experiment the brains were removed and preserved in formalin. Serial sections in the sagittal plane were cut at 35 μ of the frontal poles of the left hemispheres of seals 3 and 4, and in the horizontal plane of the right hemisphere of seal 4. Every twentieth section was stained with thionine and mounted.

RESULTS

In conformity with the rounded shape of the skull, the high position of the nostrils and the microsmatic habits of the seal, the brain tends to a more spherical form than in the terrestrial carnivores.

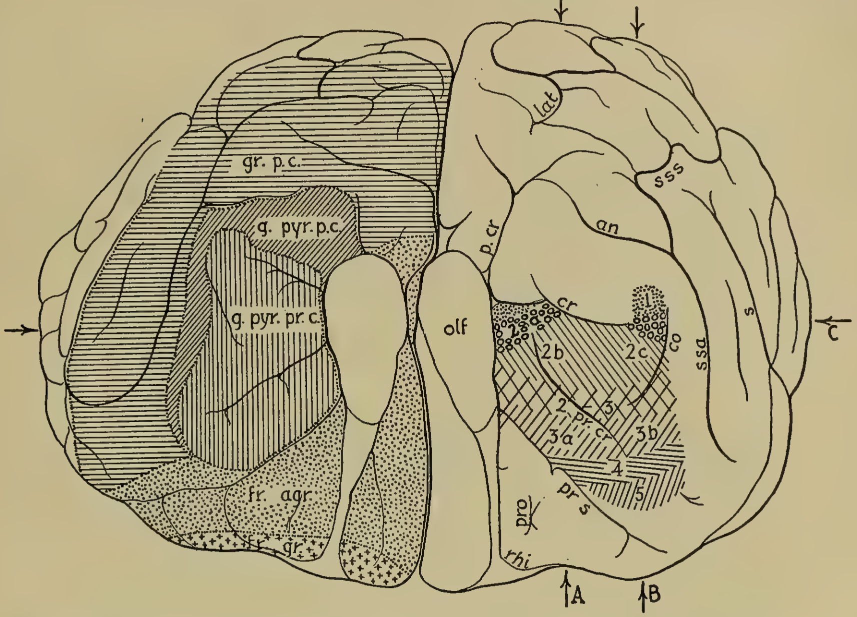


FIG. 2. Outline drawing of a frontal view of the brain of seal 4. × 1.5. The sulci are labelled as in Fig. 1.

The electrically excitable cortex is indicated on the left hemisphere. The numbers are referred to in the text.

The histological areas are indicated on the right hemisphere. *fr. agr*, Area frontalis agranularis. *fr. gr*, Area frontalis granularis. *g pyr p c*, Area gigantopyramidalis postcentralis. *g pyr pr c*, Area gigantopyramidalis precentralis. *gr p c*, Area granularis postcentralis (the caudal boundary of this area is not defined in the figure).

The general pattern of the sulci, however, was found to be similar, as illustrated in Figs. 1 and 2. A number of additional shallow sulci were present, which resulted in a reduplication and subdivision of the gyri, a condition more pronounced in other aquatic mammals (*cf.* Langworthy, 1932, 1935). Certain features in which the frontal lobe differed from that of other carnivores may be noted. The sulcus cruciatus was relatively small and was quite shallow laterally, but medially became deeper and, behind the olfactory bulb, ran into the well-developed sulcus postcruciatus. The sulcus ansatus was well marked and entered the S. suprasylvius anterior. In some instances it extended medially as a shallow groove into the S. postcruciatus, but in no case into the S. lateralis. The S. coronalis was very shallow, appearing merely as a slight depression in some of the hemispheres. In one case it extended ventrally as far as the S. precruciatus, which was also shallow. The large Gyrus proreus was divided into three parts by the S. proreus and the thin olfactory stalk.

PHYSIOLOGICAL OBSERVATIONS

The boundaries of the electrically excitable cortex under the present experimental conditions were as follows. The posterior limit was defined by the S. cruciatus. Lateral to this a small area giving movements of the tail extended onto the G. sigmoideus posterior, medial to the S. coronalis. The medial and ventromedial boundaries were sharply defined at the lateral margin of the olfactory bulb and by the S. presylvius. The lateral and ventro-lateral boundaries did not follow the superficial markings except along the posterior third of the S. coronalis, but extended well onto the G. suprasylvius anterior (see Fig. 2).

In two of the animals movements of the hind limb were obtained by stimulation of high intensity (10 volts) from the posterior lip of the S. cruciatus. In no other case, however, were striped muscle responses evoked by superficial stimulation outside of the designated area.

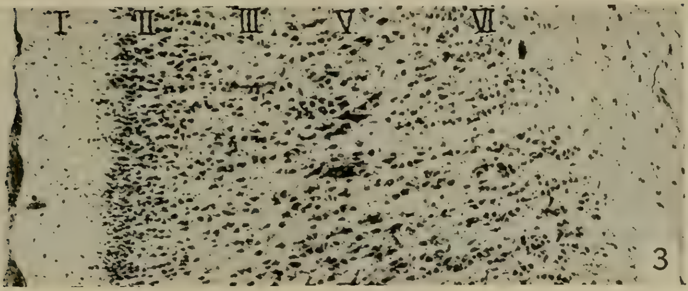
The motor cortex was readily subdivided, on the basis of the movements evoked, into the following areas: (1) tail area, (2) tail and hind-flipper area, (3) fore-flipper area, (4) neck area, and (5) face area. These are correspondingly numbered in Fig. 2.

The tail area (1) was small and sharply defined. The only movement obtained consisted of elevation and deflection of the tail to the contralateral side.

EXPLANATION OF FIGURES 3–6

FIGS. 3, 4, 5 and 6. Microphotographs of portions of a sagittal section of the left hemisphere of seal 4 in the plane indicated by arrow *A* in Fig. 2. Thionine stain. 35 μ. × 35.

3. Area gigantopyramidalis precentralis, caudal to the S. precruciatus (hind-flipper area).
4. Area gigantopyramidalis precentralis, rostral to the S. precruciatus (fore-flipper area).
5. Area frontalis agranularis, rostral to the S. presylvius.
6. Area granularis postcentralis, through the S. ansatus.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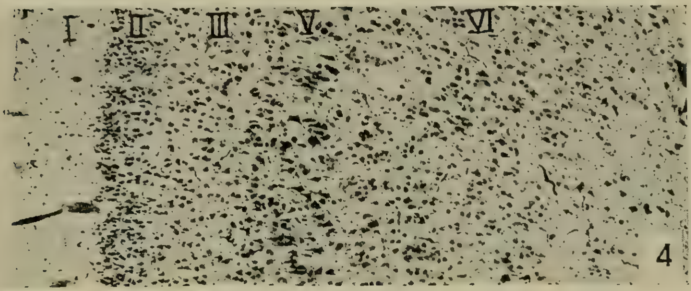
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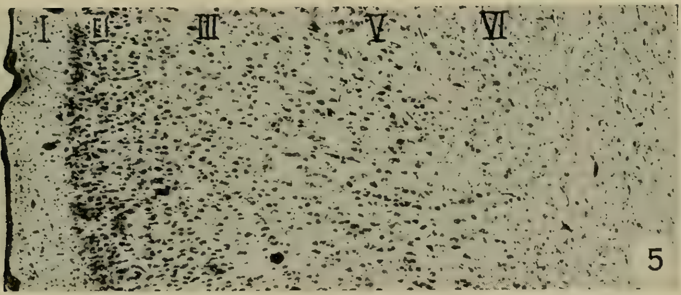
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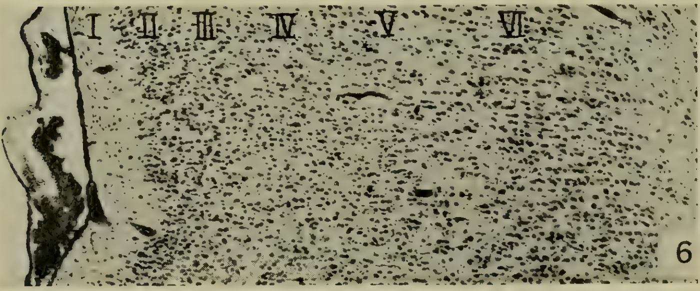
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V
VI
6

FIGURES 3–6

The tail and hind-flipper area (2*a*, 2*b*, 2*c*) was the most extensive and merged into the fore-flipper area (3*a*, 3*b*) in a zone (2–3) from which various combinations of movements of the contralateral fore-flipper, both hind-flippers and tail were evoked. From 2*a* the tail and contralateral hind-flipper were elevated and deflected contralaterally. From 2*b* and *c* similar movements resulted of the tail and both hind-flippers, together with ventral arching of the back so as to raise the hindquarters of the animal off the table. From *b* the flippers were flexed at the digits, whereas from *c* they were extended.

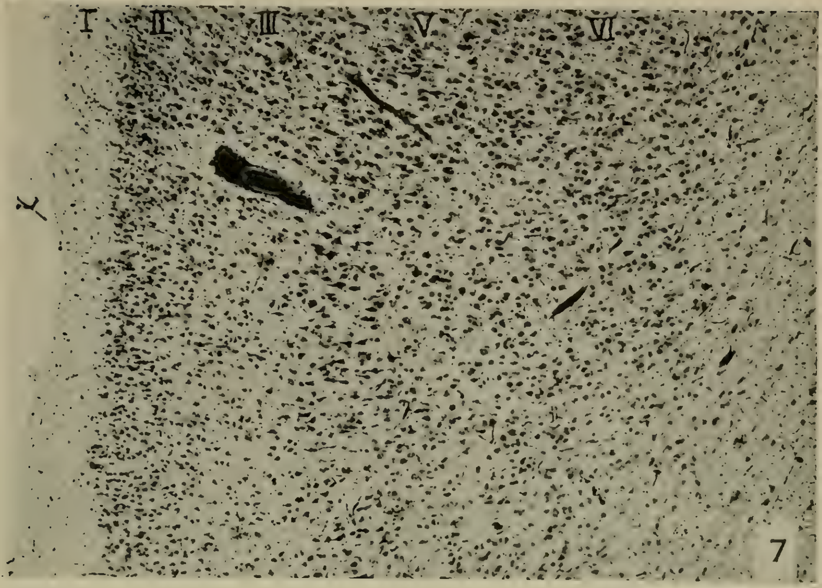
The line of demarcation between the fore-flipper and neck areas was much sharper than that between the fore- and hind-flipper areas. Movements of the contralateral, but not of the ipsilateral, fore-flipper were obtained. From 3*a* there was extension of the digits, moderate extension at the elbow and adduction at the shoulder. From 3*b* there was flexion of the digits and elbow with abduction at the shoulder.

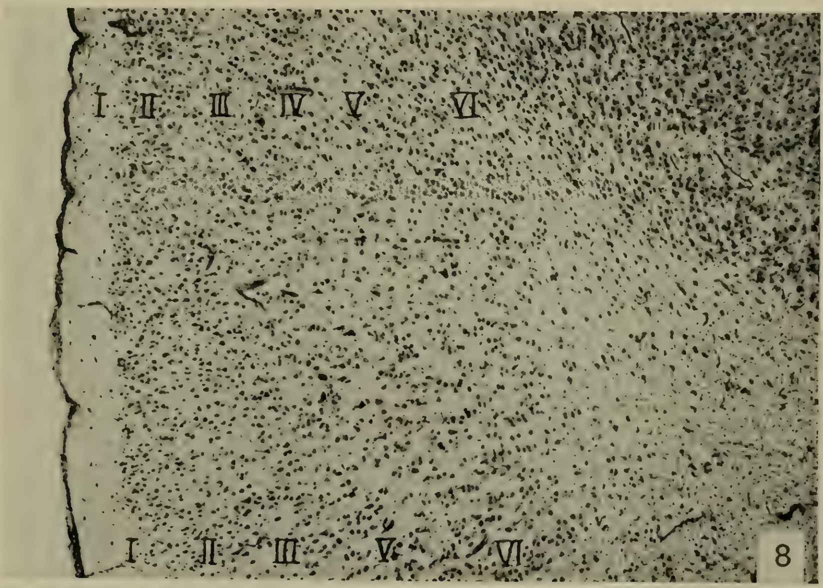
The boundaries of the neck area (4) were moderately well defined. Movements of the shoulder girdle, drawing the shoulder forward, and of the neck, with deviation of the head to the contralateral side, were obtained either alone or in combination. The shoulder was localized posterior to the neck.

The face area (5) was somewhat larger than the tail area, but much smaller than those for the flippers. Retraction of the contralateral angle of the mouth and closure of the contralateral eye, usually separately, occasionally together, were the only movements elicited.

The boundaries between areas 3 and 4, and 4 and 5 could be defined within approximately 1 mm. in a single series of stimulations, but there was a shift of the boundary of as much as 2 mm. backwards or forwards in successive series of observations depending on whether the previous stimuli had been applied in front or behind respectively. Similarly, the predominating movements evoked from the zone 2–3 depended on whether the preceding stimulation had been of the hind- or of the fore-flipper areas.

The most excitable area of the motor cortex was found in the central region of the hind-flipper area (the region of the largest giganto-pyramidal cells, see below). Here, in four of the seals, an intensity of 0.5 volts with a.c. stimulation evoked strong movements and in one case a single break-shock (Harvard inductorium, 7 cm.) resulted in a jerky contraction of the contralateral hind-flipper. Epileptiform after-discharge of varying duration was regularly elicited by a.c. stimulation of 3–5 volts for 10 to 20 seconds from any of the areas, but was most marked from areas 2 and 3.





FIGS. 7 and 8. Microphotographs of portions of sagittal sections of the left hemisphere of seal 4. Thionine stain. 35 μ. × 35.

7. Transition from the Area gigantopyramidalis precentralis (face area) to the Area frontalis agranularis, the lower border of the electrically excitable area in the plane of arrow *B*, Fig. 2.

8. Area gigantopyramidalis postcentralis and the transition to Area granularis postcentralis above, just rostral to the S. ansatus in the plane of arrow *A*, Fig. 2.

HISTOLOGICAL OBSERVATIONS

The exposed cortex of the frontal lobes was well developed and the several cell layers clearly defined. The average thickness was approximately 2 mm. In the depths of the sulci, however, it became much thinner, at the expense of the lower layers, and less well differentiated. This was particularly marked in the cruciate, precruciate and presylvian sulci.

The histological areas conformed in general to the pattern and structure described by Smith (1935) in the dog. Their extent on the exposed surface of the frontal lobe is shown in Fig. 2. Certain characteristics of the classical six-cell layers of the several areas, as seen in sagittal and horizontal sections, may be summarized as below.

Area gigantopyramidalis precentralis.—(Figs. 3, 4, 7, 9 and 10.) Layer I was better developed than in the other areas, but Layer II was relatively thinner and less dense, with many small to medium-sized pyramidal cells. Layer III was very broad, with numerous medium-sized pyramidal cells. Layer IV, absent. Layer V contained typical giant pyramidal cells which tended to be arranged in groups between fiber bundles. This layer showed more variation than did the others in different parts of the area. The cells were largest in the upper, central region (Fig. 1) (the central portion of the hind-flipper area), becoming smaller toward the periphery (Figs. 7, 9, 10) (including the tail, neck and face areas). There was a rather abrupt change from the posterior to the anterior lip of the precruciate sulcus (*cf.* Fig. 3 with Fig. 4), but elsewhere the transition was gradual. These cells were also considerably smaller around the sulci, particularly in the lower half of the cruciate sulcus, although a narrow band of gigantopyramidal cells again appeared in the posterior lip of this sulcus. Layer VI was broad, with numerous small, fusiform cells.

The myeloarchitecture was prominent throughout the area and the cells of layers III, V and VI appeared to be grouped between radiating bundles of fibers. Around the bottoms of the sulci, however, the orientation of the cells seemed to be determined by U fibers.

The boundary of the Area gigantopyramidalis precentralis and the

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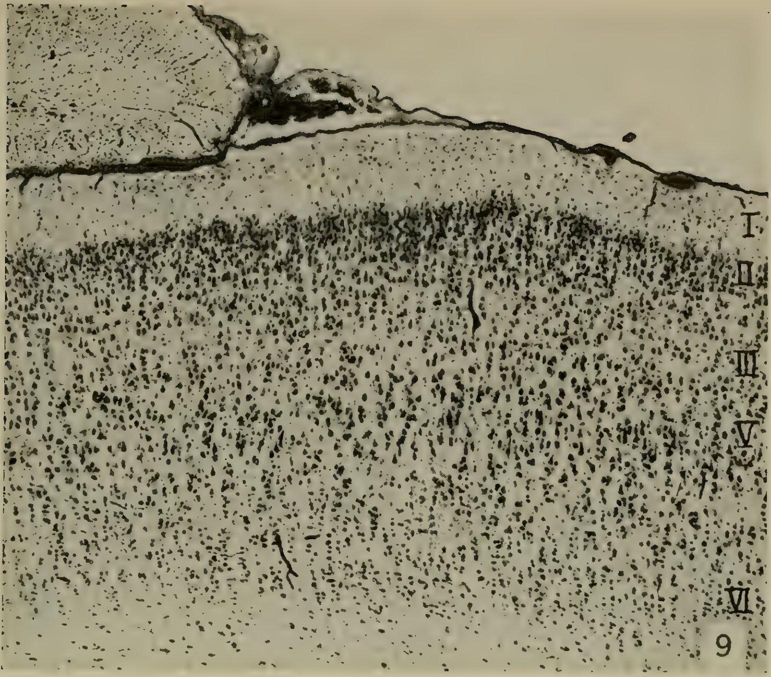
EXPLANATION OF FIGURES 9 AND 10

FIGS. 9 and 10. Microphotographs of portions of a horizontal section through the right hemisphere of the brain of seal 4 in the plane indicated by arrow *C*, Fig. 2. Thionine stain. 35 μ. × 35.

9. Transition from the Area gigantopyramidalis precentralis (right), to the Area frontalis agranularis (left), at the lateral margin of the Bulbus olfactorius (upper left).

10. Transition from the Area gigantopyramidalis precentralis (extreme left) through the Area gigantopyramidalis postcentralis (center) to the Area granularis postcentralis (extreme right) between the S. coronalis and the S. suprasylvius anterior.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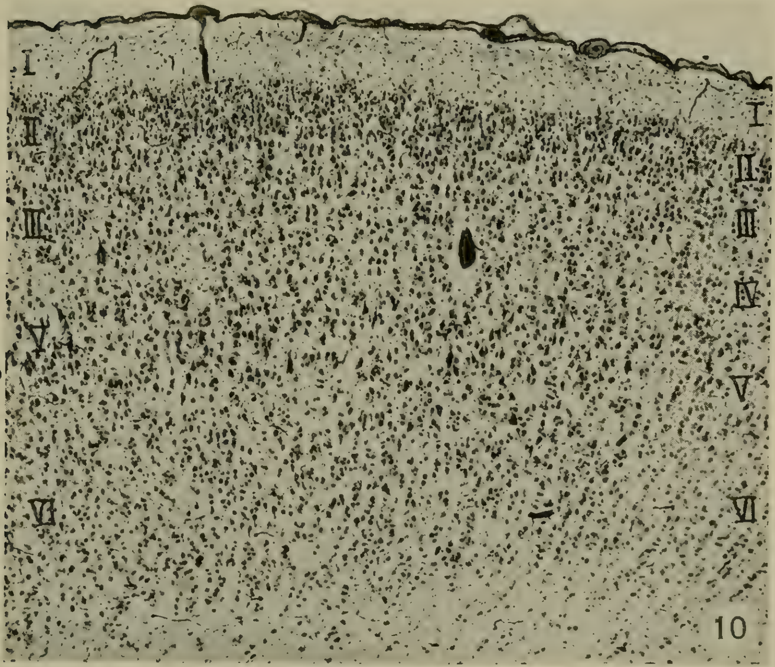
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FIGURES 9 AND 10

Area frontalis agranularis was quite sharp (Figs. 7 and 9). The transition, however, to the Area gigantopyramidalis postcentralis (Fig. 8) and to the granular cortex of the G. suprasylvius anterior was very gradual (Fig. 10).

Area frontalis agranularis.—(Figs. 5 and 7.) In comparison with the precentral gigantopyramidal cortex this area showed the following characteristics. Layer I was approximately half as thick. Layer II contained more cells, but with a smaller proportion of pyramidal forms. Layer III also showed an increase in number of cells. Layer IV was absent. Layer V varied in thickness at different regions and the cells varied in size from slightly smaller to slightly larger than those of layer III. Occasional cells contained heavily staining Nissl substance, but the majority were relatively pale. Layer VI was similar to that in the area gigantopyramidalis. The myeloarchitecture was less prominent, although in places the radiating arrangement of cells between fiber bundles was noticeable.

This type of cortex extended over a wide area, including most of the G. proreus and the ventral portions of the G. genualis where a transition to a granular type of cortex occurred posteriorly. Anterior to this area, between it and the undifferentiated cortex of the basal olfactory areas, was the Area frontalis granularis. The latter was poorly defined and small in extent. It was somewhat thicker, but the cell types were similar with the addition of the granular cells of layer IV.

Area gigantopyramidalis postcentralis.—(Figs. 8 and 10.) The following changes occurred through the transition zone between this and the Area gigantopyramidalis precentralis. Layer I was reduced to about one-third as thick. Layer II increased in density and breadth, most of the cells being rounded in form. Layer III was considerably reduced and the cells became smaller. Layer IV appeared as a narrow, ill-defined band of granular cells. The giant pyramidal cells of layer V persisted, but greatly reduced in numbers and size. Layer VI showed little change. The radiating bundles of fibers through the lower layers remained prominent.

This area covered the greater part of the exposed surface of the G. sigmoideus posterior, the transition to the Area granularis postcentralis being sharply defined at the postcruciate and ansate sulci (Fig. 9). A similar, though narrower, zone extended round the lateral border of the Area gigantopyramidalis precentralis, between it and the granular cortex of the G. suprasylvius anterior (Fig. 10).

Area granularis postcentralis.—(Figs. 6 and 8.) This area differed strikingly from those described above. Layer I was narrow. Layer II was broad and dense, the cells being mainly small and rounded. It appeared to fuse with layer IV, which contained similar cells.

Layer III showed as a band of scattered, small to medium-sized, pyramidal cells amongst the granular cells at the junction of layers II and IV. Layer V was sharply defined, consisting chiefly of fibers, with a few cells of the small, pyramidal type. Layer VI was well developed and for the most part contained rounded cells with occasional fusiform and triangular elements.

The decrease in thickness of this type of cortex round the base of the sulci was much less marked than in the other areas and was mostly due to diminution in layer VI.

This area extended caudally over the G. lateralis and rostrolaterally over the G. suprasylvius anterior. In the latter location layer V was better developed than it was caudally. Laterally it was bounded by an area in the anterior wall of the S. suprasylvius anterior which showed a poorly developed layer III, a narrow, irregular layer IV, and sparsely scattered, but very large, pyramidal cells in layer V. Rostral to the Area gigantopyramidalis precentralis the transition to the Area frontalis agranularis was relatively sharp.

DISCUSSION

The present experiments were performed under a single form of anæsthesia and on animals otherwise normal. Because of these limitations it is obvious that conclusions with regard to correlations between so-called electrical excitability and the cytoarchitecture of the different cortical areas are of little or no significance (*cf.* Tower, 1936; Rioch and Rosenblueth, 1935). It is of interest, however, that the lowest threshold was found in the region of the largest Betz cells; and the observation that a strong movement could be evoked by a single shock in this area is unusual in carnivores under dial anæsthesia.

The extent and differentiation of the Area gigantopyramidalis, on the basis of both the physiological and anatomical observations, compare favorably with these features in other carnivores as observed by the present author under similar conditions of anæsthesia (dog and cat) and as described in the literature (*cf.* Langworthy, 1928; Smith, 1933, 1935, etc.). In contrast with this high degree of development of the central representation stands the apparently simpler and less differentiated form of the extremities of the seal. It may be concluded that the extent of central representation is to be correlated with function and not with form. Further evidence for this hypothesis is furnished by the following considerations. In the terrestrial carnivores electrical stimulation of the cortex evokes movements of the extremities which resemble certain phases of the normal activity of the animal when initiating locomotion, feeding, seizing prey, etc. In the seal, however, the movements of the trunk and extremities elicited from the cortex resembled parts of the normal swimming actions, which are finely and swiftly executed, and not the clumsy movements of locomotion of that animal on land.

The rostral position of the gigantopyramidal area and the shallowness of the S. cruciatus may be due in part to the relatively small size of the olfactory brain, allowing more room for superficial development of the frontal lobes of the hemispheres.

The cerebellum, particularly the lateral lobes, and the pons were found to be very large in the seal as compared with the dog and cat, resembling the development of these structures in other aquatic mammals (Langworthy, 1932, 1935). This is probably to be correlated with the wide extent of the Area frontalis agranularis. The teleological explanation which suggests itself is the necessity for particularly fine coördination of movements controlling the position of the animal in three-dimensional space.

Summary

The exposed cortex of the frontal lobes of the left hemispheres of six young seals (Phoca vitulina) under dial anæsthesia was stimulated electrically. Movements of the tail, both hind-flippers and the contralateral fore-flipper, neck and face were evoked. The cortical localization is charted in Fig. 2.

Histological examination (thionine stain) of three frontal lobes revealed a well-developed cortex divisible into cytoarchitectural areas structurally similar to those in other carnivores, but differing in their extent and their relation to the sulci (Fig. 2).

On the basis of a comparison of the seal with the terrestrial carnivores it is concluded that the degree of differentiation of the cortical representation is to be correlated with function and not with form.

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